Université de Montréal

Faculty of Medicine

Environmental tobacco smoke exposure and risk for Crohn's disease in children

Ali Lalavi

Biomedical Sciences Program

Université de Montréal

Mémoire submitted to the faculty of Medicine of Université de Montréal, in order to obtain the master's degree in Biomedical Sciences, General Option

July 2016

Résumé

L'importance des déterminants génétiques de la maladie de Crohn (MC) chez l'enfant est bien connue, mais nos connaissances sur la contribution des facteurs de risque environnementaux demeurent limitées. Parmi les facteurs de risque du déclenchement de la MC chez l'adulte, figure le tabac. Le lien entre le tabagisme actif et le déclenchement de la MC a été maintes fois démontré. Cependant, les études menées jusqu'à présent sur l'influence de la fumée secondaire sur le déclenchement de la MC chez l'enfant ne sont pas consistantes, et ont souvent montré des résultats contradictoires. Le principal objectif de notre étude était donc de déterminer l'influence de l'exposition à la fumée secondaire pendant la grossesse et durant l'enfance sur le déclenchement de la MC chez l'enfant.

Méthodes: Nous avons mené une étude cas-témoins auprès d'enfants caucasiens. Les cas avaient reçu un diagnostic de MC avant l'âge de 20 ans à la clinique de gastroentérologie pédiatrique du CHU-Sainte-Justine de Montréal (n=132), et les témoins (n=131) ont été sélectionnés parmi les patients du service de gastroentérologie ou d'orthopédie du même hôpital, sans histoire de maladie chronique intestinale. Nous avons apparié les cas et les témoins selon le moment du diagnostic (± 3 mois) et leur lieu de résidence (à l'aide du code postal). L'information sur l'exposition à la fumée secondaire au cours de la grossesse et durant l'enfance, ainsi que les autres facteurs de risque ont été colligés à l'aide d'un questionnaire. L'analyse des déterminants du déclenchement de la MC a été faite par régression logistique pour estimer le ratio de cote (RC) ainsi que les intervalles de confiance correspondant (IC95%).

Résultats: L'âge moyen (± ET) des cas était légèrement plus élevé que celui des témoins (12,7 ± 4,0 vs. 11,4 ± 4,7; p=0,01). Le sexe était réparti de manière égale entre les groupes. L'histoire familiale s'est avérée significativement associée à la MC (p=0,01). La régression logistique multivariée n'a montré aucun lien statistiquement significatif entre le tabagisme de la mère pendant la grossesse et la MC, en comparant les mères qui ont fumé pendant la grossesse avec celles qui n'ont fumé ni pendant la grossesse

ni après l'accouchement (RC= 1,55 ; IC95% = 0,84-2,86). Le tabagisme chez le père non plus ne semble pas augmenter le risque de la MC chez l'enfant (RC= 0,95 ; IC95% = 0,33-2,75). Bien que durant l'enfance, le tabagisme chez la mère et l'exposition à la fumée secondaire semblent augmenter le risque de la MC, les résultats ne sont pas statistiquement significatifs (RC= 3,54 ; IC95% = 0,71-17,57). Par contre, le tabagisme chez le père durant l'enfance augmente significativement le risque du déclenchement de la MC (RC= 2,52 ; IC95% = 1,11-5,72) et ce particulièrement quand les parents avaient fumé durant la grossesse.

Conclusions: L'exposition à la fumée secondaire durant la grossesse ne semble pas influencer le risque du déclenchement de la MC chez l'enfant. Cependant, durant l'enfance l'exposition à la fumée secondaire, particulièrement quand le père est fumeur, devient déterminante et contribue au risque du déclenchement de la MC. D'autres études sont nécessaires pour mieux élucider ces liens.

Mots clés : fumée secondaire, maladie de Crohn, pédiatrie, risque environnemental

Abstract

While the genetic contributors to pediatric-onset Crohn's disease (CD) have been well identified, there is limited information on the putative environmental risk factors. In adult-onset CD, active smoking has been consistently shown to be positively associated with the disease. In children, there is interest in understanding whether passive exposure to environmental tobacco smoke (ETS) could confer similar risks. However, current studies have provided inconsistent results. The major objective of our study was thus to comprehensively ascertain whether ETS exposure during pregnancy and childhood was associated with the risk of developing CD in children.

Methods: We carried out a case-control study based on Caucasian children diagnosed with CD (n=132) prior to age 20 at a pediatric gastroenterology clinic in Montreal (CHU-Sainte-Justine). Controls (n=131) were children having visited the orthopedic or gastroenterology clinics, who did not have a past/current history of IBD, were diagnosed within ± 3 months of case diagnosis and resided in the same geographic area (based on the first 3 digits of the postal code) as the cases. Information on ETS during and post-pregnancy and other potential risk factors for CD was acquired using a structured questionnaire. Associations between ETS and CD were analyzed using unconditional logistic regression. Odds ratios (OR) and corresponding 95% confidence intervals (95% CI) were estimated.

Results: The mean age (±SD) of the cases 12.7 (±4.0) was slightly higher than the controls (11.4±4.7) (p-value=0.01). Gender was equally distributed between the groups. Family history was positively associated with CD (p-value=0.01). Multivariate logistic regression did not reveal any association with CD when mothers who smoked during pregnancy were compared to those who neither smoked during pregnancy nor post-pregnancy (OR=1.55, 95% CI=0.84-2.86). Paternal smoking during pregnancy was also not associated with risk of CD (OR=0.95, 95% CI=0.33-2.75). Exposure of ETS to the child during childhood via maternal smoking appeared to increase risk (OR=3.54, 95% CI=0.71-17.57) but the risks were not

significant. Paternal smoking during childhood also appeared to enhance risk of CD, in particular when the parents also smoked during pregnancy (OR=2.52, 95% CI=1.11-5.72).

Conclusions: ETS exposure *per se* during pregnancy does not seem to confer risks of CD in children. However, ETS exposure during childhood either from maternal or paternal smoking appears to contribute to risk of CD in the child. Further studies are required to validate these associations.

Key words: Crohn's disease, tobacco smoking, children, environmental risk factors

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

CD: Crohn's disease

CI: confidence interval

ETS: environmental tobacco smoke

GI: gastrointestinal

IBD: inflammatory bowel disease

IC: indeterminate colitis

OR: odds ratio

SMR: standardized mortality ratio

TNF: tumor necrosis factor

UC: Ulcerative Colitis

ACKNOWLEDGEMENTS

I would like to thank my thesis supervisor, Dr Devendra Amre, for his guidance. I would also like to extend my gratitude to the CHU Ste-Justine Research Center and the Ste-Justine Foundation, as well as the Department of Biomedical Sciences of the University of Montreal, for their generous support.

INTRODUCTION

Crohn's disease (CD) and ulcerative colitis (UC), which make up a collection of disorders known as inflammatory bowel diseases (IBD), affect approximately 1 in 150 Canadians. Canada has the highest reported rates of IBD in the world, with Quebec having one of the highest rates in the country (Fedorak et al, 2010). Once thought to be a rare condition among children and adolescents, recent estimates from a population-based, province-wide study in Canada indicate that the incidence of CD among children between 9 and 20 years of age now approaches that in adults (Bernstein et al, 2006).

CD presenting in the paediatric age group presents numerous challenges, which differ from those facing adults. The disease is more severe in children and they are susceptible to more complications and surgical interventions (Griffiths et al, 2004).

Although the precise aetiologies of both disorders are unknown, genetic predisposition, in combination with environmental risk factors, are thought to contribute to their development (Baron et al, 2005; Tysk et al, 1988; Loftus et al, 2004). Family studies and, more recently, genome-wide association studies have shown an important role of genetic predisposition to these diseases (Jostins et al, 2012). However, the absence of these diseases among monozygotic twins, the absence of a family history in the majority of cases, and the evolution of disease incidence in developed nations have all heightened the importance of environmental factors in disease aetiology.

The one factor consistently associated with adult-onset CD is active smoking. CD patients are more likely to be smokers; this association is supported by data indicating that smokers have higher relapse rates and more aggressive disease, suggesting that an element of tobacco smoke exacerbates disease (Bernstein et al, 2006). On the other hand, active smoking has been shown to be protective for UC. The divergent roles of tobacco smoke as a deleterious factor in CD and a protective agent in the development of UC have been a source of debate over the past three decades. The exact mechanisms of these opposing effects have yet to be resolved (Ananthakrishnan, 2015).

Though tobacco exposure is a confirmed risk factor for adult-onset CD, whether tobacco exposure during childhood is a risk factor for childhood-onset CD remains unclear (Aspberg et al, 2006). Approximately twenty-five percent of Quebec women between 20 and 40 years of age are smokers, indicating a high potential for environmental tobacco smoke (ETS) exposure to influence disease development in children (Dubois et al, 2005). However, while some studies have suggested that ETS exposure to the foetus or newborn, either during pregnancy or post-pregnancy may be positively associated with CD in childhood (Lashner et al, 1993; Russell et al, 2005; Roberts et al, 2011), others do not support such an association (Rigas et al, 1993; Baron et al, 2005). Most of the inconsistency in previous studies is likely due to lack of comprehensive data on ETS exposure during different time-periods of development and/or small sample sizes. Considering the high prevalence of childhood exposure to ETS in Quebec, studying its role in CD is of the utmost importance. In the subsequent sections we present a detailed review of the epidemiology of CD, highlight current research on the links between ETS exposure and CD, and present a rationale for the purpose of our study.

I) LITERATURE REVIEW

1. CROHN'S DISEASE: AN OVERVIEW

Inflammatory bowel disease (IBD) is comprised of two major disorders: ulcerative colitis (UC) and Crohn's disease (CD) (Silverberg et al, 2005). IBD is characterized by symptomatic flare-ups alternating with periods of disease inactivity (Cohen, 2003). UC is a chronic inflammatory condition characterized by relapsing and remitting episodes of inflammation limited to the mucosal layer of the colon. It almost invariably involves the rectum and typically extends in a proximal and continuous fashion to involve other portions of the colon. CD is characterized by transmural inflammation and by skin lesions. The transmural inflammatory nature of CD may lead to fibrosis and strictures, and to obstructive clinical presentations that are not typically seen in UC. More commonly, the transmural inflammation results in sinus tracts, giving rise to micro-perforations and fistulae (Gasche et al, 1998). Classification of IBD facilitates clinical decisions, discussions with the family, eligibility for clinical trials, and epidemiologic research. This classification is usually accomplished with the combination of endoscopy and imaging of the upper gastrointestinal tract. The distinction between the two types of IBD is not always obvious, as some patients may present with characteristics of both UC and CD. If the disease type remains uncertain after complete evaluation, the term "indeterminate" colitis is used (Silverberg et al, 2005). IC (indeterminate colitis) makes up 10-15% of cases (Geboes et al, 2008). Some newer classification schemes suggest using the term "colonic IBD, type unclassified", reserving "indeterminate colitis" for patients in whom the type of IBD remains uncertain after colectomy and pathological evaluation (Silverberg et al, 2005).

SYMPTOMS OF CD

The clinical manifestations of CD are more variable than those of ulcerative colitis. Patients can have symptoms for many years prior to diagnosis (Farmer et al, 1975). Fatigue, prolonged diarrhoea with abdominal pain, weight loss, and fever, with or without gross bleeding, are the hallmarks of CD (Pimentel et al, 2000) (Mekhjian et al, 1979). CD symptoms alternate between periods of activity and periods of

remission. CD and UC share a number of extra-intestinal manifestations generally related to inflammatory disease activity (Burgmann et al, 2006; Ziv et al, 2015).

Table 1- Extra-intestinal manifestations of inflammatory bowel disease

Common extra-intestinal manifestations
Musculoskeletal
Arthritis colitic type, ankylosing spondylitis, isolated joint involvement such as sacroilitis Hypertrophic osteoarthropathic clubbing, periostitis, metastatic Crohn's disease Miscellaneous osteoporosis, aseptic necrosis, polymyositis, osteomalacia
Skin and mouth
Reactive lesions: erythema nodosum, pyoderma gangrenosum, aphthous ulcers, vesiculopustular eruption, necrotizing vasculitis, Sweet syndrome, metastatic Crohn's disease Specific lesions: fissures and fistulas, oral Crohn's disease, drug rashes Nutritional deficiency: acrodermatitis enteropathica (zinc), purpura (vitamins C & K), Glossitis (vitamin B), hair loss and brittle nail (protein) Associated diseases: vitiligo, psoriasis, amyloidosis, epidermolysis bullosa acquisita
Hepatobiliary
Specific complications: primary sclerosing cholangitis (PSC) and bile duct carcinoma, small duct PSC, cholelithiasis Associated inflammation: autoimmune chronic active hepatitis, pericholangitis, portal fibrosis and cirrhosis, granuloma in Crohn's disease Metabolic: fatty liver, gallstones associated with ileal Crohn's disease

Ocular
Uveitis iritis, episcleritis, scleromalacia, corneal ulcers, retinal vascular disease, gastrobulbar neuritis, Crohn keratopathy
Metabolic
Growth retardation in children and adolescents, delayed sexual maturation
Less common extraintestinal manifestations
Blood and vascular
Anemia due to iron, folate, or B12 deficiency or autoimmune hemolytic anemia, anemia of chronic disease, thrombocytopenic purpura, leukocytosis and thrombocytosis, thrombophlebitis and thromboembolism, arteritis and arterial occlusion, polyarteris nodosa, Takayasu arteritis, cutaneous vasculitis, anticardiolipin antibody, hyposplenism
Renal and genitourinary tract
Urinary calculi (oxalate stones in ileal disease), local extension of Crohn's disease involving ureter or bladder, amyloidosis, drugrelated nephrotoxicity Renal tubular damage with increased urinary excretion of various enzymes (eg, beta-N-acetyl-D-glucosaminidase)
Neurological
Up to 3 percent of patients may have noniatrogenic neurologic involvement, including peripheral neuropathy, myelopathy, vestibular dysfunction, pseudo-tumor cerebri, myasthenia gravis, and cerebrovascular disorders. Incidence equal in ulcerative colitis and Crohn's disease. These disorders usually appear 5 to 6 years after the onset of inflammatory bowel disease and are frequently associated with other extra-intestinal manifestations.

Airway and parenchymal lung disease
Pulmonary fibrosis, vasculitis, bronchitis, acute laryngo-tracheitis, interstitial lung disease, sarcoidosis. Abnormal pulmonary function tests without clinical symptoms are common (up to 50 percent of cases).
Cardiac
Pericarditis, myocarditis, endocarditis, and heart block (more common in ulcerative colitis than in Crohn's disease); cardiomyopathy, cardiac failure due to anti-TNF therapy Pericarditis may also occur from sulfasalazine/5aminosalicylates
Pancreas
Acute pancreatitis: more common in Crohn's disease than in ulcerative colitis. Risk factors include 6-mercaptopurine and 5-aminosalicylate therapy, duodenal Crohn's disease
Autoimmune
Drug induced lupus and autoimmune diseases secondary to anti-TNF-alpha therapy Positive DNA, anti-double stranded DNA, cutaneous and systemic manifestations of lupus

Modified from: Das KM. Relationship of extra-intestinal involvements in inflammatory bowel disease: New insights into autoimmune pathogenesis. Dig Dis Sci 1999; 44:1.

The reasons for the variable disease course in CD are not completely known. There is a dysregulation of pro-inflammatory cytokines towards Th1 cell predominance along with mesenchymal cell and fibroblast proliferation. This is accompanied by an altered expression of adhesion molecules and co-stimulatory molecular species and supplemented by an altered production of protective mucosal mucins, weakening the mucosal barrier and enabling chemically induced mucosal injury (Ziv et al, 2015). The symptoms are most probably due to a chronic inflammation caused by an abnormally permeable gut

wall (Baumgart et al, 2012). This allows the antigens to cross the epithelial cell lining of the GI (gastrointestinal) tract and to produce a reaction from the underlying immune system. The permeability of the gut wall is related to faulty tight junctions (proteins that are supposed to render the space between the epithelial cells of the GI tract impenetrable) (Baumgart et al, 2012). Repetitive inflammation of the GI tract results in lesions along its walls, which consecutively, may cause the symptoms associated with CD (Cosnes et al, 2011). Inflammation can also cause more serious damage, for instance, a narrowing of the GI tract (stricture), swelling of the gut wall (abscesses) or abnormal passages between different regions of the GI tract (fistulas) (Cosnes et al, 2011). However, there is no evidence that the severity of symptoms are interrelated to the degree of damage done to the GI tract wall (Cosnes et al, 2011).

Inflammation involves the intestinal wall full-thickness, and extends to mesenteric fat and lymph nodes. In the early stages, lesions typically manifest as cryptic abscesses and aphthous ulcers. The chronicity of inflammation leads to the onset of non-caseating granulomas that, although representing the histological hallmark of CD, are present in fewer than 50% of endoscopic biopsies and in 70% of surgical specimens, since they localize more often in the submucosa than in the mucosa. Other common features are lymphoid aggregates in the submucosa, abundant lympho-monocytic infiltrates in the lamina propria, and mucosal fissures, which eventually become real penetrating fistulas through the gut wall. Aphthous ulcers, which are initially small and superficial, converge and surround areas of unaffected mucosa giving the gut mucosa the typical ‘‘cobblestone’’ appearance. Possible complications are intra-abdominal or intra-parietal-perianal abscesses, fistulas, linking the gut with another intestinal tract or a contiguous organ (e.g., bladder, ureter, and vagina) or the skin, and strictures, which are caused by intestinal fibrosis and lead to the subsequent development of pre-stenotic dilatations (Sabatino et al, 2011).

A consensus hypothesis is that, in genetically predisposed individuals, both exogenous factors (e.g., composition of normal intestinal microbiota) and endogenous host factors (e.g., intestinal epithelial cell barrier function, innate and adaptive immune function) interact to cause a chronic state of

dysregulated mucosal immune function that is further modified by specific environmental factors (e.g., smoking, enteropathogens). Although chronic activation of the mucosal immune system may represent an appropriate response to an unidentified infectious agent, a search for such an agent has thus far been unrewarding in IBD. As such, IBD is currently considered an inappropriate immune response to the endogenous commensal microbiota within the intestines, with or without some component of autoimmunity. Importantly, the normal intestines contain a large number of immune cells in a chronic state of so-called physiologic inflammation, in which the gut is restrained from full immunologic responses to the commensal microbiota and dietary antigens by very powerful regulatory pathways that function within the immune system (e.g., FoxP3+ T regulatory cells). During the course of infections in the normal host, full activation of the gut-associated lymphoid tissues occurs, but is rapidly superseded by dampening of the immune response and tissue repair. In IBD, this process may not be regulated normally (Harrison, 2011).

DIAGNOSIS OF CD

Due to the complex nature of the disease and absence of a gold standard for evaluation, CD cannot be diagnosed by a single test (Ferkolj et al, 2008). Therefore, a combination of multiple tests is used (Ferkolj et al, 2008) (Morrison et al, 2009). A medical history and physical exam will provide descriptive characteristics of symptoms and their frequency and severity (Baumgart et al, 2012). The diagnosis of this group of disorders, particularly small bowel disease, has proven considerably difficult in the past, due to a myriad of clinical presentations, and the paucity of diagnostic tests to effectively evaluate the small bowel. The recent evolution in diagnostic modalities holds great promise in overcoming these limitations of the past. Novel techniques including laboratory tests (serologic and fecal markers), endoscopic modalities (capsule endoscopy and double-balloon enteroscopy), radiologic studies

(CT enterography, MR enterography, CT colonography (CTC), and MR colonography (MRC)), and endoscopic mucosal imaging techniques (magnification endoscopy, chromoendoscopy, confocal laser endomicroscopy, and optical coherence tomography (OCT)) represent significant advancements both in the diagnosis and long-term management of IBD (Shabana et al, 2011). Biopsies of the GI tract can help to detect lesions and other pathologies of the intestinal wall (Baumgart et al, 2012). Endoscopy, more specifically ileo-colonoscopy, is a more invasive technique normally used in CD diagnosis. It permits the visualization of lesions, abscesses and fistulas in the GI tract. Endoscopy combined with biopsies is the current gold standard of CD diagnosis (Baumgart et al, 2012) (Morrison et al, 2009). The use of imaging techniques, both as a diagnostic tool and as a tracking instrument, for disease progression is becoming ever more popular, as the field is continuously evolving (Al-Hawary et al, 2012). Imaging techniques, including computed tomography enterography and magnetic resonance enterography, let the specialists evaluate a cross-sectional image of the bowel, instead of being limited to the superficial mucosal layer of the gut, as seen with more traditional endoscopy (Al-Hawary et al, 2012).

When the CD diagnosis has been established, further classification of the disease according to the Montreal, Rome or Vienna classification systems will help in determining the suitable therapy and course of action. These classification systems are based on the structural location, the type, the severity of the intestinal damage, and the demographic characteristics of the patient (Baumgart et al, 2012) (Morrison et al, 2009).

TREATMENT OF CD

Because no curative therapies are available for CD, treatment objectives include slowing the course of disease and treating the symptoms by repairing the damage caused to the GI tract wall (strategy denoted as “mucosal healing”) (Baumgart et al, 2012) (De Cruz et al, 2013). The primary end-points of therapy in CD are induction of remission, maintenance of remission and management of complications.

Therapeutic approaches are different according to disease location (ileocecal or colonic disease), the presence of complications (fistula, abscesses, strictures) and disease severity (Sabatino et al, 2011).

The disease progression varies from one CD patient to another, hence, careful monitoring of the disease phenotype is critical in maximizing the benefits of therapy (Cosnes et al, 2011) (Baumgart et al, 2012) (Vermeire et al, 2012). The disease phenotype, in addition to patient characteristics, for instance age at CD onset, the site and the behaviour of the disease and medical history, may be used to predict prognosis and to adapt the treatment to the patient. The most recent classification system, the Paris system, was established to improve treatment of paediatric IBD by containing additional patient characteristics when classifying the disease phenotype (Vermeire et al, 2012).

To regulate the inflammation, medical therapy is used: steroids or anti-TNF (tumour necrosis factor) agents, either as mono- or combination therapy (Baumgart et al, 2012). During the last decade, anti-TNF agents and the emergence of new therapeutic concepts have dramatically changed IBD management, especially in the early phases of the disease. Salicylates remain the therapeutic basis in UC, while their efficacy in CD has not been confirmed. A rapid step-up approach is considered for managing early-phase IBD by providing early immunomodulators such as immunosuppressant and anti-TNF in case of poor disease course. Some specific situations (severe, extended or complicated forms) require the most efficient first-line therapy consisting of a combination of anti-TNF and immunosuppressant (Poullenot et al, 2014). Fast acting drugs, like steroids, are usually combined with slower acting drugs, like immunotherapy drugs (Baumgart et al, 2012) (Morrison et al, 2009). Different types of medical therapies are chosen based on the nature and the severity of the symptoms, associated illnesses and personal factors (Baumgart et al, 2012).

Surgery is reserved for specific indications described below (Schwart, 2015). The majority of CD patients (70-80%) require surgery within 20 years of diagnosis, and the majority of patients undergoing

surgery will experience recurrence of the disease (Cosnes et al, 2011) (De Cruz et al, 2013). The percentage of patients whose endoscopy results stay normal 10 years post-surgery is less than 5% (Cosnes et al, 2011). The risk of recurrence is related to tobacco use, the extent of the damage to the GI tract wall and surgical history (Baumgart et al, 2012). In addition, nutritional support in the form of aggressive enteral regimens or, if necessary, parenteral nutrition is used to manage the malnutrition that is common in patients with CD (Schwart, 2015).

Biologic therapies are new advances in CD management. They involve the use of drugs, which target specific components of the inflammation process, including drugs that bind TNF-alpha (Morrison et al, 2009). Recently, two new antibodies have been approved: golimumab, a new option for UC, and vedolizumab, with another more selective mechanism of action, which could be useful for UC as well as CD. Ustekinumab is an alternative treatment option for refractory CD (Gomollón, 2014).

Antibiotics are often used in the treatment of CD patients with fistulas or perianal changes. The most common are ciprofloxacin and metronidazole, but there are also studies characterizing efficient IBD treatment with rifaximin and ornidazole. They also increase the likelihood for remission when combined with budesonide (Sobczak et al, 2014).

2. PAEDIATRIC CROHN'S DISEASE

The age distribution at CD onset is bimodal. The first peak arises in the early twenties and a second peak occurs between the ages of 50 and 70. Cases diagnosed before adulthood (<20 years) approximately represent 25% of all cases (Kim et al, 2004) (Karlinger et al, 2000). The incidence of Crohn's in children is twice that of UC (Rabizadeh et al, 2013). There are around 5900 or more Canadian children under the age of 18 with IBD (Gouvernement de Québec, 2003). An individualized therapeutic strategy in a child with IBD is necessary in terms of both medical and psychosocial management. Special attention should be paid towards growth, immunizations and mental health (Rabizadeh et al, 2013). Depression and anxiety are particularly prevalent and have a multifaceted aetiology; including IBD-related factors such as cytokines and steroids used to treat IBD and psychosocial stress (Szigethy et al, 2010). IBD is a disorder with potential morbidities and lifelong challenges; hence, understanding the different entities that affect children with the disorder can improve overall care (Rabizadeh et al, 2013).

Children with IBD are more likely than adults to present with extraintestinal manifestations (aphthous ulcers, joint involvement, and growth delay being the most common) (Huang et al, 2014). Children with IBD are more likely than adults to present with extensive disease, both in CD and UC. Diagnosis requires a high index of suspicion, as children may present with less typical signs, such as poor growth and delayed puberty. In very young patients with IBD, the paediatric clinician must consider a broader range of immunological and allergic disorders (Lev-Tzion et al, 2012).

GROWTH FAILURE:

A unique aspect of paediatric IBD is the issues related to growth (Rabizadeh et al, 2013). Growth failure rates, commonly defined as height below the third percentile, were ranged from 10 to 56% at the time of CD diagnosis (Abraham et al, 2012). Forty percent of children with CD have growth failure compared to less than 10% of UC patients. In fact, evidence of impaired linear growth may be the only presenting sign of IBD and can precede gastrointestinal symptoms. Growth failure is likely secondary to chronic malnutrition due to inadequate intake, excessive losses and increased energy requirement, as well as the effects of inflammation on growth (Rabizadeh et al, 2013). Nutritional deficiencies, notably insufficient vitamin D levels, lead to bone demineralization (Kim et al, 2004). Interestingly, patients appear to have normal growth hormone levels, but insulin-like growth factor (IGF) 1 is reduced, suggesting hormone insensitivity, possibly secondary to inflammation instead of deficiency. Medication can play a role in growth failure as well. Recurrent and chronic administration of high-dose corticosteroids may lead to decreased collagen production and, hence, a decrease in linear growth (Rabizadeh et al, 2013). Moreover, the immunological imbalance could perturb the normal release of growth hormones (Kim et al, 2004).

QUALITY OF LIFE:

Despite the physical issues of IBD, the disease also imposes a psychosocial burden on children. Compared with healthy children, paediatric patients with IBD can have behavioural and emotional functioning issues, particularly depression and anxiety, social functioning, and self-esteem. Depression and anxiety are rampant in children with IBD. Symptoms of depression and/or anxiety have been noted in 25 to 30% of children with IBD, and 10 to 30% meet the criteria for clinical depression or an anxiety disorder (Rabizadeh et al, 2013). The deteriorated quality of life manifests itself through family problems,

socialization difficulties, medical adherence problems, and missing school and extracurricular activities, in addition to depression and anxiety (Bousvaros et al, 2006).

BONE HEALTH:

Children with IBD may develop osteopenia as a result of inflammatory cytokine production, malnutrition, malabsorption or inadequate intake of calcium and vitamin D, prolonged inactivity and/or corticosteroid therapy. When compared to controls, children with IBD, especially those on prolonged courses of corticosteroids, may be at increased risk for fractures (Rabizadeh et al, 2013).

IMMUNIZATIONS:

The long-term treatment for IBD involves the use of anti-inflammatory agents and immunosuppressive medications including steroids, anti-metabolites and biologic therapies. IBD patients are considered immunocompromised as a result of these treatments (Banaszkiewicz et al, 2015). Protection against vaccine-preventable illnesses is critical in paediatric IBD patients. However, the safety and efficacy of immunizations must be considered before recommending their administration in these patients. With the exception of those with live agents (measles, mumps, rubella; varicella; influenza intranasal spray), vaccines can be safely administered in IBD patients on immunosuppressants. Hence, immunization in paediatric and adult IBD patients should not deviate from the recommended schedules in the general population (Rabizadeh et al, 2013). Attenuated vaccines are contraindicated in patients treated with immunosuppressive drugs (GKS at high dose, immunomodulators, biological drugs) during the entire treatment period and up to 12 weeks following the cessation of therapy. Immunosuppressive therapy can be initiated 4–6 weeks after the administration of attenuated vaccines (Banaszkiewicz et al, 2015).

3. EPIDEMIOLOGY OF CD

TEMPORAL TRENDS

In the past century, a substantial rise in the global incidence of CD has been reported (Economou et al, 2008). The annual incidence of CD varies from 0 to 20.2 per 100,000 in North America and 0.3 to 12.7 per 100,000 in Europe, with a prevalence of 37.5 to 248.6 per 100,000 and 4.9 to 505 per 100,000, respectively. However, this incidence has changed substantially in the past several decades. Within countries considered to have a high incidence of IBD, some populations, such as the First Nations population in Canada or the Arab Bedouin population in Israel, have a markedly reduced incidence when compared to the general population. The risk of IBD is threefold higher in the Jewish population than in non-Jewish populations. Furthermore, the risk of IBD is higher, particularly among Ashkenazi populations (compared to Sephardim populations), and American and European Jewish populations compared to those residing in Israel. Additional evidence comes from immigration studies. Children of immigrants coming from developing to developed countries display a greater risk of CD than their parents, further providing evidence for an environmental factor (Benchimol et al, 2011; Ng et al, 2013). An initial report by Probert et al. identified the incidence of UC in first-generation and second-generation Indian migrants to the UK to be similar to the native UK population, and higher than the incidence in the countries of origin, whilst the incidence of CD was lower. Subsequent studies from the UK and Sweden suggested that the increase in risk was most apparent in the second generation, whereas the first-generation immigrants from low incidence countries continued to have lower risk than those from the country migrated to. In British Columbia, Canada, the incidence of paediatric IBD among immigrant South Asians was even higher than in the native white population. In one of the largest studies examining the effect of immigration on disease risk (Benchimol et al., 2011), authors found a markedly lower risk of IBD in immigrants, particularly from East Asia, than in the general population of Ontario, Canada. Older age at immigration was associated with a greater reduction in risk of IBD and the decreased risk persisted in children from East Asia, Central Asia and Latin America, but not those from the Middle East, South

Asia, Africa, or Western Europe. The phenotype of IBD in emerging populations and with migration also seems more distinct and milder than in established Western populations, though to what extent this phenomenon is a reflection of the natural history of disease compared with differences in health-seeking behaviour, and patient and provider preferences is unclear. The immigrant Indian population in the UK and the native population in India and the rest of Asia have markedly lower rates of surgery than Western countries. Foreign-born Hispanic individuals in the United States had lower rates of surgery or less use of biological therapy than non-Hispanic white individuals (Ananthakrishnan, 2015). Though the presence of a genetic component in CD is well established, genetic predisposition alone cannot explain the speed at which the disease progresses. Therefore, there must be an underlying environmental triggering factor, most probably linked to industrialization and the modern society.

Canada has one of the highest incidence rates of CD and UC in the world (Bernstein et al, 2006). Based on incidence data collected from 1998 to 2000, the national averages were 13.4 and 11.8 cases of CD and UC per 100,000 person-years, respectively. With a national population estimated to be 34 million in April 2010, there were approximately 4500 new cases of CD and 4000 new cases of UC diagnosed in Canada in 2010 (Fedorak et al, 2010). Globally, in terms of CD incidence, Canada is second to New Zealand (16.5 cases per 100,000 population) and has a slightly higher rate than Scotland (11.7 cases per 100,000 population) and England/Wales (5.9 to 11.1 cases per 100,000 population). The rates for the United States, Denmark and Sweden range from 7 to 8.9 cases per 100,000 population (Economou et al, 2008).

With respect to pediatric patients (0 to 19 years of age), the average incidence rates of CD and UC in 5 Canadian provinces (Alberta, British Columbia, Manitoba, Nova Scotia and Saskatchewan) were 8.32 and 4.34 cases per 100,000 population, respectively. In July 2009, there were 654 and 341 reported new cases of paediatric CD and UC, respectively. In Quebec, the annual incidence rate of CD in youth 0 to 19 years of age from 1993 to 2002 was 13.9 cases per 100,000 person-years (Lowe et al, 2009). By

combining the incidence data for CD in these six provinces, the mean incidence rate was 9.25 cases per 100,000 person-years, resulting in an additional 73 cases per year for a total of 727. The Canadian incidence rates for both paediatric CD and UC are very high compared to those reported for northern California (USA) in 2010, which were 2.7 and 3.2 cases per 100,000 children, respectively (Abramson et al, 2010). Similar to the rankings for CD and UC incidence rates in adults, Canada also has one of the highest prevalence rates for pediatric-onset CD and UC in the world, with 374 and 456 cases per 100,000 population, respectively (Statistics Canada, 2010). Within the United States, the rates are only 201 and 238 cases per 100,000 population for CD and UC, respectively (Kappelman et al, 2007). Northern European rates range from 27 to 48 per 100,000 population for CD, and 58 to 157 per 100,000 population for UC (Bernstein et al, 2006). Canadian rates are even higher when compared to countries from southern regions such as Spain, Italy, Cuba and South America, which have rates less than1.0, and 1.5 to 5.8 per 100,000 population for CD and UC, respectively (Bernstein et al, 2006).

The occurrence of paediatric CD also seems to have increased over the past 40 years (Kim et al, 2004). In a 2011 review of tendencies in international incidence rates of paediatric CD, 60% of the studies surveyed reported a significant increase in disease occurrence (Benchimol et al, 2011).

GEOGRAPHICAL TRENDS

The map below, taken from the 2012 Molodecky systematic review of worldwide IBD prevalence and incidence, displays CD occurrence and prevalence rates since 1980, by region (Molodecky et al, 2012).

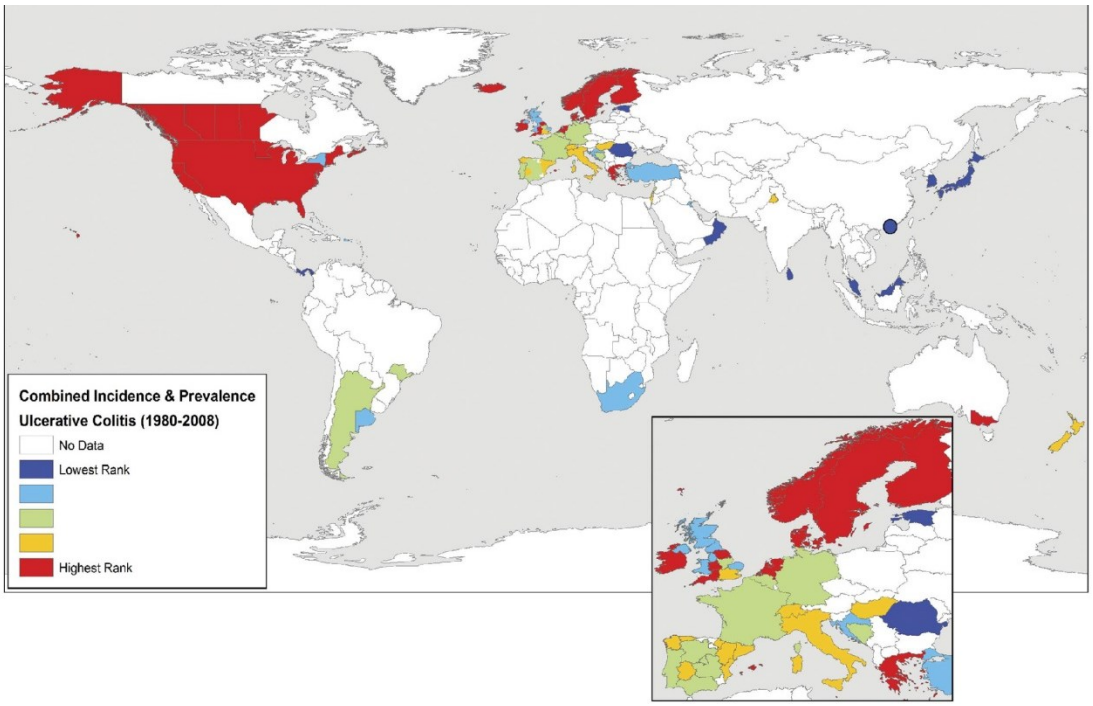


Figure 1: CD incidence rate by geographical area, taken from Molodecky et al., 2012

The figure shows the variation in disease incidence and prevalence among diverse regions of the world (Molodecky et al, 2012). High incidence rates are concentrated in developed countries, particularly, North America, Europe and Australia. Developing regions, such as Asia, Africa and South America have much lower rates. These results are consistent with the findings of the previous literature review, conducted by Economou et al. in 2008 (Economou et al, 2008).

It is essential to note that most IBD epidemiology studies were conducted in developed countries in Northern Europe and North America (Ng et al, 2013) (Molodecky et al, 2012). The lack of data collected from other countries might partially explain the differences in incidence.

The geographic trends observed in adult CD are very similar to the paediatric CD population, as seen in the following figure from Benchimol et al, 2011.

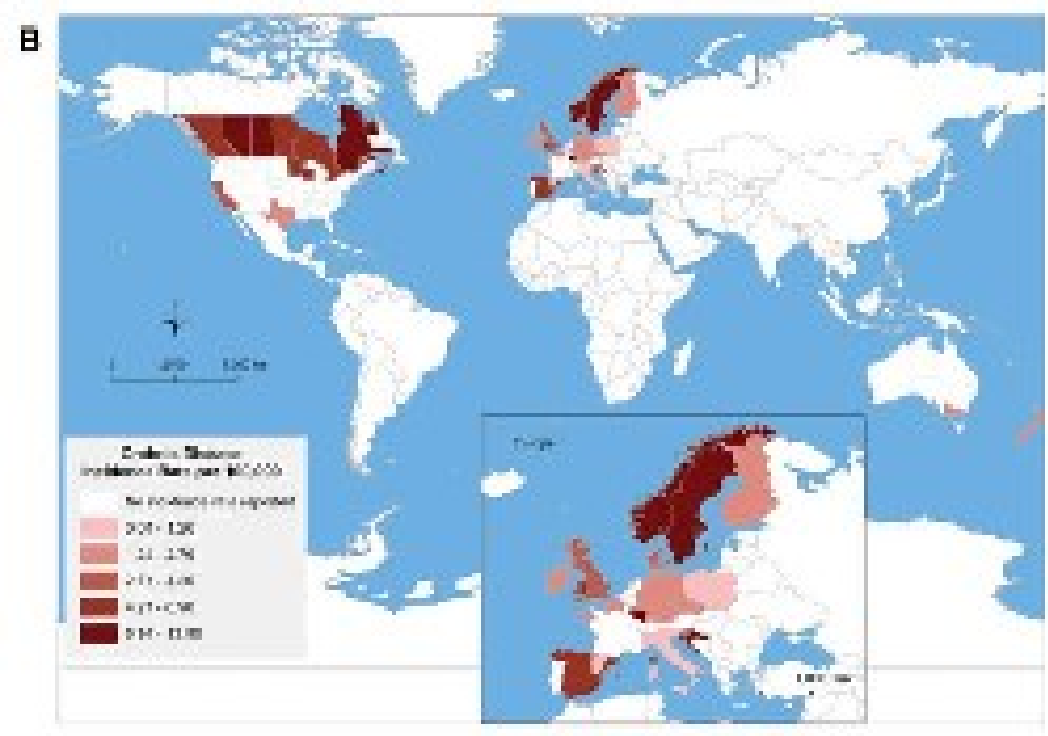


Figure 2: Pediatric CD incidence rate by geographical area, taken from Benchimol et al., 2010 (incidence rates from 1990)

A noticeable difference has been observed in North-South CD incidence rates in Europe; the CD incidence is greater in the region North of the Alps (7/100 000) compared to the South (3.9/100 000) (Economou et al, 2008) (Castiglione et al, 2012). A similar trend has also been observed in individual countries; for instance, northern France and Scotland display higher incidence rates than their southern regions (Ng et al, 2013). In Canada, a West-East difference has been perceived, with lower rates in British Columbia and the highest rates in Quebec and Nova Scotia (Lowe et al, 2009). The origin of these gradients is still not clear. Hypotheses consist of differences between urban and rural regions, and asymmetrical immigration (Molodecky et al, 2012; Lowe et al, 2009).

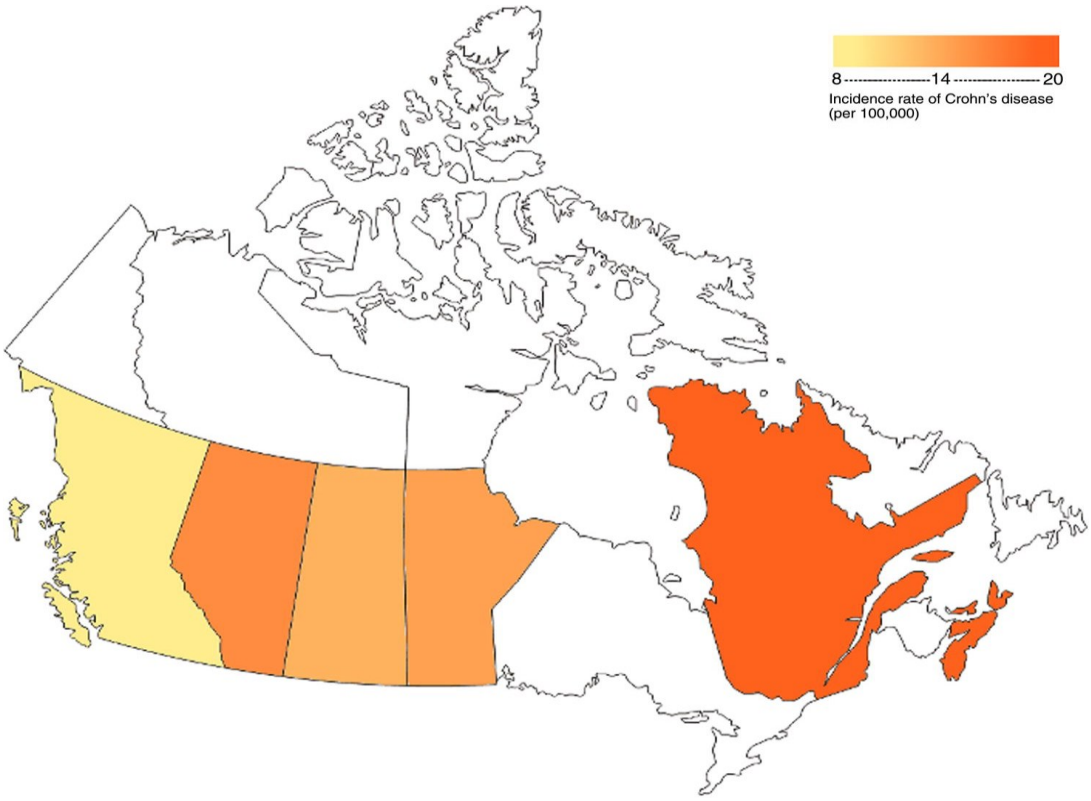


Figure 3: Variation of incidence rates for CD within Canada showing an East–West disease gradient (Siew C Ng et al, 2012)

The incidence of CD remains low in Asian, Southern and Eastern European and developing countries, but it is gradually increasing. This trend is particularly apparent in Asia (Ng et al, 2013). For instance, in Japan, CD incidence has increased from 2.9/100 000 in 1986 to 13.5/100 000 in 1998. Similarly, South Korea has seen a rise in CD incidence from 7.6/100 000 in 1997 to 30.9/100 000 in 2005 (Ng et al, 2013). The rising trend in these regions is following the model of the developed countries from100 years ago; the UC incidence rate increased first, followed by the CD incidence (Ng et al, 2013).

From 2000 to 2006, the annual mean mortality rate, primarily due to CD and UC, was 75 and 39 deaths per year, respectively (Statistics Canada, 2010). Compared to deaths in Canada due to all causes, CD and UC deaths collectively accounted for 0.05% of the annual total. Although the annual mortality

rate for both CD and UC has been slowly increasing, the ratio of these deaths to deaths due to all causes has been constant at 0.05%. An independent meta-analysis of 13 studies found that CD patients have an age-adjusted risk of premature death that is 50% greater than the general population (standardized mortality ratio [SMR] 1.52; 95% CI 1.32 to 1.74; P<0.0001) (Canavan et al, 2007). Each year, 114 deaths in Canada are attributed to CD and UC (Fedorak et al, 2010). The economic costs of IBD are estimated to be $2.8 billion in 2012 (almost $12,000 per IBD patient). Direct medical costs exceed $1.2 billion per annum and are driven by the cost of medications ($521 million), hospitalizations ($395 million) and physician visits ($132 million). Indirect costs (societal and patient costs) total $1.6 billion and are dominated by long-term work losses of $979 million. Compared to the general population, the quality of life patients experience is low across all dimensions of health (Rocchi et al, 2012)

4. CD RISK FACTORS

CD is the result of both genetic and environmental factors (Carbonnel et al, 2009). There is recognition that the genetic risk factors do not act in isolation but in synergy with the external environment as well as the internal 'environment', namely the gut microbiota. The development of IBD is thought to be governed by a series of interactions between these three spheres, which simultaneously not only increase the complexity of disease pathogenesis, but also offer several avenues for intervention and improvement of patient outcomes (Ananthakrishnan, 2015).

GENETIC PREDISPOSITION

Family history is one of the strongest risk factors for CD. For instance, in a matched case-control study investigating potential risk factors for IBD, family history was found to be the most important prognosticator of the disease (OR: 4.6, 95% CI 2.6-8.3) (Baron et al, 2005). Monozygotic twins have a 50% concordance risk for Crohn's, which is significantly higher than the concordance rate of dizygotic twins (estimated at 3%); moreover, children of parents with Crohn's have a 33% risk of developing the disease (Baumgart et al, 2012) (Rabizadeh et al, 2013) (Frolkis et al, 2013) (Ananthakrishnan, 2013) (Brant et al, 2007).

Ethnicity has also been associated with a higher risk of CD. For example, the occurrence is especially higher in the Caucasian and the Ashkenazi Jewish population (Cohen, 2003; (Karlinger et al, 2000; Cho et al, 2011). The ethnic and familial factors indicate the existence of a genetic predisposition for CD, since gene alleles are distributed differently across populations (Cho et al, 2011). Having a single relative with confirmed IBD increases the risk of CD (OR: 3.1; 95%CI: 2.2–4.3) and UC (OR: 2.5; 95%CI: 1.9–3.5). The 'dose effect' was confirmed when multiple family members had IBD: CD (OR: 7.4; 95%CI: 3.4–16.1) and UC (OR: 6.8; 95%CI: 3.1–14.9) (Leong, 2010). The first CD-associated gene was described in 2001, and subsequent Genome-wide association studies (GWAS) and meta-analyses

have identified a total of 163 susceptibility loci for IBD, with 140 for CD (Jostins et al, 2012). In the past 5 years, researchers have identified several monogenic forms of severe early-onset colitis. For example, single mutations in IL10 (interleukin-10) and the genes encoding its receptor (IL10RA and IL10RB), as well as mutations in XIAP (X-linked inhibitor of apoptosis protein), have been shown to cause severe early-onset IBD (Peterson et al, 2014).

In accordance with the polygenic model for disease, a number of susceptibility loci associated with CD have been identified. The gene for NOD2/CARD15 (caspase activation recruitment domain), an important protein in innate immunity, was one of the first associated risk alleles for Crohn's (Bonen et al, 2003). In a case-control study, the CD population attributable risk for CARD15 was estimated at 46.8% (Brant et al, 2007). There is a 20 to 40 fold increased risk of developing disease if a person has two risk alleles. The genetic loci identified in patients with Crohn's implicate many biologically relevant immune pathways such as IL-23, IL-17 and IL-10. For the most part, though overlap exists, Crohn's genes variations appear to be in pathways involved in microbe recognition and immune system responses such as autophagy, while in UC, genes appear to be involved in intestinal barrier integrity and function. In infants, one genetic mutation of significant interest is found in the interleukin-10 (IL-10) pathway. This rare autosomal recessive mutation leads to an infantile form of severe IBD that sometimes requires bone marrow transplantation (Rabizadeh et al, 2013).

Other genes have also been associated with CD. For instance, mutations of the ATG16L1 and IRGM genes, that play a role in the pathogen-degradation process, cause disturbance in the autophagy pathway (Spalinger et al, 2013).). The NOD2 gene has a role in peptidoglycan recognition, which are particles found on invading bacteria, and its polymorphisms significantly increase the risk of CD (Cho et al, 2011). However, these genes and other genetic loci identified thus far explain less than a third of CD cases, supporting and reinforcing the role of environmental factors and/or gene-environment interactions in the etiology of CD (Amre et al, 2003, Amre et al, 2002)

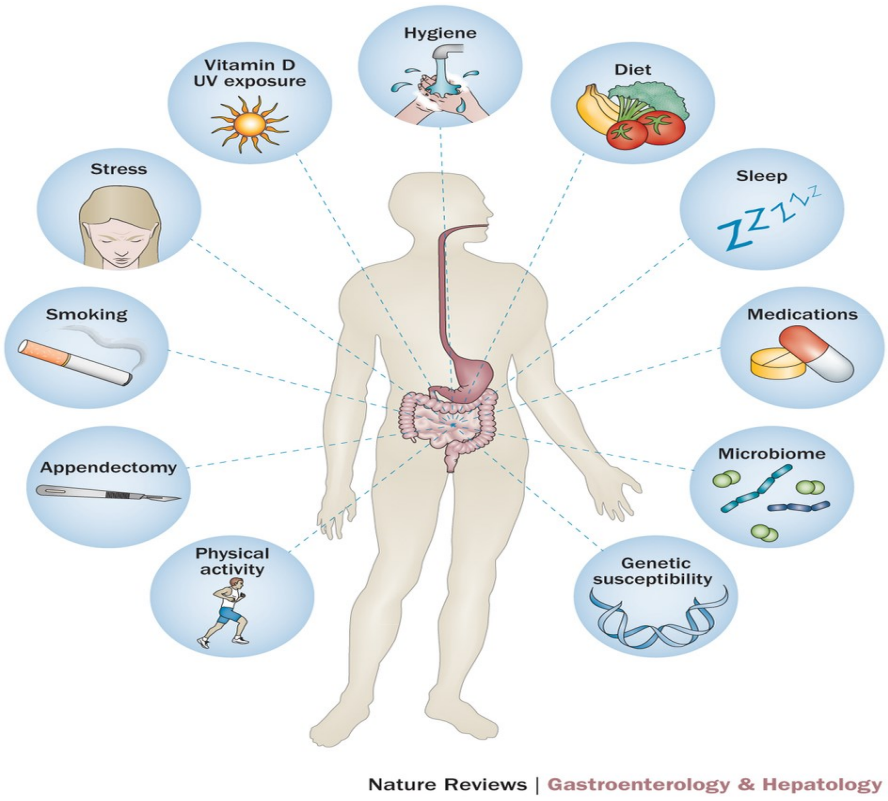


Figure 4: The interaction between genetics, immunology, environment and microbiome.

Many factors may participate in the development of IBD, such as genetic, immunological and environmental, including diet, depression, stress and the influence of free radicals. As depicted in Figure 6, IBD is thought to develop from genetic predisposition (leading to immunological abnormalities), dysbiosis of the gut microbiota and environmental influences. No single risk factor is sufficient for disease development and the complex interactions among factors lead to the development of IBD (Ananthakrishnan, 2013).

Urban living and lack of exposure to pets and vegetable gardens have been hypothesized to be associated with an increased risk of IBD. These factors all seem to be crucial for the development of IBD, each to a different degree. Studies with monozygotic twins clearly indicate the possibility of the existence

of environmental factors in IBD development. Another investigation on monozygotic twins revealed that, despite identical genomes, they differ in microflora. However, it was also revealed that genetic factors determine the composition of the microflora and are responsible for maintaining homeostasis in the intestine (Sobczak et al, 2014).

Dietary fibre (particularly fruits and vegetables), saturated fats, depression, impaired sleep, and low vitamin D levels have all been associated with incident IBD. Interventional studies assessing the effects of modifying these risk factors on natural history and patient outcomes are an important unmet need (Ananthakrishnan, 2015).

Oral contraceptives are known risk factors for CD. In two large prospective cohorts of women, authors observed a significant association between oral contraceptive use and risk of CD (Ananthakrishnan, 2013). Compared to women with no history of oral contraceptive use, the age-adjusted HRs for CD were 2.88 (95% CI 1.69 to 4.89) among women currently using oral contraceptives, and 1.50 (95% CI 1.13 to 1.99) among past users (Khalili et al, 2012). This effect seemingly increases with the number of years of oral contraceptives use (Ng et al, 2013). Stress, socioeconomic status (SES), diet and the use of antibiotics are among other risk factors of CD, while breastfeeding and sunlight (vitamin D) are believed to be protective (Ng et al, 2013) (Frolkis et al, 2013) (Ananthakrishnan, 2013) (Green et al, 2006). Nonetheless, research findings for these factors are still inconsistent and their association with CD is yet to be clearly established.

The role of stress in IBD also seems to be significant. Stressful conditions induce the activation of the hypothalamic–pituitary–adrenal (HPA) axis, which inhibits the immune system, impairs digestive functions and may induce a mucosal and systemic inflammatory reaction in IBD patients (Sobczak et al, 2014).

Limited physical activity (fewer than two sporting activities per week) in childhood was a risk factor for both CD and UC according to one study. A study by Persson et al. (1990) revealed that the relative risk (RR) of CD was inversely related to regular physical activity: for weekly and daily exercise, the estimated RRs were 0.6 (95% CI 0.4–0.9) and 0.5 (95% CI 0.3–0.9), respectively (Hlavaty et al, 2013).

Infrequent contact with animals in childhood (defined as less than once per week) was another independent risk factor for CD (OR 1.7). A case–control study from Canada on 581 patients with IBD and 433 controls also revealed that contact with animals, particularly cats, was protective in CD (OR 0.66, 95% CI 0.46–0.96) (Hlavaty et al, 2013).

SMOKING AND CD

ACTIVE SMOKING AND CD

The dramatic increase in the incidence of inflammatory bowel disease (IBD), especially CD, in developed countries over the second half of the 20th century highlights the important role that environmental factors play in the pathogenesis of IBD. Cigarette smoking is one of the most well documented environmental risk factors for adult-onset IBD (Jones et al, 2008). Among all the 534 studies conducted on CD risk factors, the association between smoking and IBD has gathered the most persuasive evidence (Bernstein et al, 2006). Oddly, smoking seems to have a protective effect against UC, but is a risk factor for CD (Ananthakrishnan, 2013). Based on a recent review, being a current smoker doubles one's risk of CD (OR 2.0; 95%CI: 1.48–2.68), whereas the risk of UC is reduced (OR: 0.67; 95%CI: 0.48–0.94) (Ng et al, 2013).

Not all cohorts have identified an effect of smoking on IBD. In an Israeli study, smoking cessation was associated with an increased risk of UC, but not of CD. By contrast, smoking was associated with early age of onset and more frequent need for immunosuppressive therapy in CD among women, but not men (Reif et al, 2000).

PASSIVE SMOKE/ETS EXPOSURE & IBD

Environmental Tobacco Smoke (ETS)

ETS consists of two forms of smoke from the burning of tobacco products:

1. Side-stream smoke, or smoke that is emitted between the puffs of a burning cigarette, pipe, or cigar
2. Mainstream smoke, or the smoke that is exhaled by the smoker. When a cigarette is smoked, about one-half of the smoke generated is side-stream smoke. This form of smoke contains most of the same carcinogenic and toxic agents that have been identified in the mainstream smoke inhaled by the smoker, but at greater levels.

The health consequences of "passive smoking," or inhaling environmental tobacco smoke, have received considerable attention. The 1986 Surgeon General's report dealt exclusively with passive smoking and concluded that "involuntary smoking is a cause of disease, including lung cancer, in healthy non-smokers (Sandier et al, 1992). Children are more susceptible to the harmful effects of ETS. They can be exposed to tobacco smoke not only in their homes, but also in schools, restaurants, child-care settings, cars, and other public places. Home exposure is the most common type of exposure. Parental smoking in the home is known to lead to substantial maternal and fetal ETS exposure, subsequently affecting fetal and child health (Seong et al, 2008). A world-wide survey carried out in schools from WHO member states demonstrated that approximately 50% of the students were exposed to ETS in their homes (Warren et al, 2008). Not surprisingly, such a high exposure corresponds with the numerous health effects

reported. A recent comprehensive review (Cao et al, 2015) identified links between ETS exposure and upper and lower respiratory diseases, infections, cardiovascular disease, inflammation, and neurobehavioral deficits. Worldwide efforts to establish interventions to reduce ETS are ongoing.

Harries et al., (1982) were the first to suggest that a relationship might exist between passive smoke exposure and the development of IBD in adults. In a case-control study carried out in the UK, they acquired information on smoking from patients with UC (n=250), CD (n=192) and non-IBD controls (those visiting the fracture clinics of the same hospital) (n=230). Controls were matched to UC patients for age and gender. They observed that approximately 30% of UC patients belonged to smoking households, compared to 40% in CD and 50% in controls. These findings suggested that ETS exposure may be protective for UC. Although the study had a large sample size, details on ETS exposure were not specific. Furthermore, potential confounding from socio-economic status was not controlled for. A number of studies have subsequently examined this association. Persson et al., (1990) carried out a case-control study in Stockholm, Sweden, in which they identified patients with UC or CD from hospital discharge sheets maintained in a population-based registry, diagnosed between 1980-1984. Patient diagnosis was confirmed using medical records. An age-sex stratified random population-based sample of controls with age and sex distribution comparable to the IBD patients was recruited. After accounting for exclusions and refusals, 152 CD, 145 UC and 305 controls provided information on active and passive smoking (postal questionnaire and follow-up by telephone). ETS exposure was ascertained via the question: “How many people smoked regularly in your home during your childhood (0-15 years old)?”. They reported elevated risks for CD in adulthood (OR=1.5, 95% CI=1.0-2.3), but not for UC (OR=0.98, 95% CI=0.60-1.5). The study had many strengths. It was population-based and had a large sample. Diagnosis of IBD was confirmed. The response rates were sufficiently high (~80% across all 3 study groups). The major limitations were the potential for recall bias, non-specific measurement of ETS, and not controlling for of active smoking and other potential confounders such as SES. Furthermore, given

that only hospitalized patients with listed phone numbers were included, selection bias was a possibility. The findings for CD were not replicated in later studies by Thompson et al., (1995) (ETS exposure: regular smoking by either parent during childhood; OR=1.0, 95% CI=0.88-2.3), Elaikim et al., (2000) (CD=261, controls=430; ETS exposure: passive smoking in parental home; OR=0.8, 95% CI=0.5-1.1), Feeney et al., (2002) (CD=139, controls=139; ETS exposure: one or both parents smoking; OR=1.1, 95% CI=0.7-1.9), and Bernstein et al. (2006) (OR=0.8, 95% CI=0.6-1.2). However, Mahid et al. (2007) reported positive associations (CD=377, controls=384; ETS exposure: mother, father or other household member smoking; OR=2.0, 95% CI=1.3-3.3). Though all of the above studies also examined UC, none found any associations with ETS. Thus, there seems to be no association between ETS exposure and UC for adult-onset IBD; however, the findings are inconsistent for CD (Table 2).

Given the susceptibility of children to ETS exposure, they may be predisposed to the development of IBD. Some case-control studies have addressed this possibility with regards to two specific categories of passive smoke exposure: prenatal exposure due to maternal smoking during pregnancy and passive smoke exposure during childhood. As both of these exposures are relatively common in North America (Tong et al, 2009), investigating the risk of such ETS for childhood IBD is relevant. Lashner et al. (1993) carried out a hospital-based case-control study in Chicago, USA. They recruited 39 UC and 33 CD patients and 72 controls (friends of the patients). All participants were non-smokers. They defined post-natal ETS exposure as: smoking more than 5 cigarettes by a parent or sibling in the home at the time of symptom onset. They reported positive (but not statistically significant) associations with CD (OR=2.00, 95% CI=0.5-8.0) and UC (OR=1.7, 95% CI=0.7-4.3). When ETS exposure at birth (in the home) was examined, increased risks for IBD were noted (OR=3.02, 95% CI=1.28-7.1) with greater risks for CD than UC. Similarly, risks for IBD were increased with maternal smoking at birth (OR=2.09, 95% CI=1.02-4.3). Although different measures of ETS were examined, adding to the strength of the study, the results were likely to be overestimates given the small sample

sizes. Furthermore, the results were not adjusted for potential confounding of family history of IBD and SES. Moreover, it was unclear whether the associations with ETS exposure at birth were independent of those at symptom-onset and vice-versa. The effects of ETS exposure during pregnancy were also not assessed. Rigas et al. (1993) carried out a case-control study in New York, wherein they identified IBD patients from the medical records of patients diagnosed in hospitals from 1986 to 1990. Controls were patients without IBD, seen at the same pediatric gastroenterology departments at the respective hospitals. Information on maternal smoking and other potential risk factors was abstracted from the medical records. A total of 68 CD, 39 UC and 202 controls were examined. No association between maternal smoking and IBD was found. This study had some limitations. Information on maternal smoking was acquired from the medical records; information that may be incomplete or inaccurate. In addition, it was not possible to establish the timing of the ETS exposure from maternal smoking (whether at the time of IBD diagnosis, during pregnancy or during childhood). Furthermore, the study was likely not powered to detect potential associations. Another study carried out in Northern France by Baron et al. (2005) examined passive smoking by parents or caregivers along with a host of other potential risk factors. This case-control study was comprised of children diagnosed with IBD prior to age 17, identified from the EPIMAD population-based registry. Controls were randomly selected from the population using random digit dialling and matched for age, gender and area of residence. In total, 222 CD, 60 UC and 282 controls were interviewed via telephone. No association between passive smoking during pregnancy and IBD was evident (OR=0.84, 95% CI=0.55-1.3). This study was a comprehensive population-based study. The analysis accounted for potential confounding variables, including SES. Nonetheless, the measurement of ETS was likely imprecise and did not cover the appropriate susceptibility time windows. A separate analysis for CD was carried out. The latter is relevant as smoking is negatively associated with UC, and the results may be underestimated by the inclusion of these patients. A frequency distribution of the ETS variable among the subjects was not presented, precluding a clear interpretation of their negative findings.

The Baron et al. (2005) study was followed by a case-control study conducted in South East Scotland among children with early onset IBD, where cases of IBD diagnosed at less than 16 years of age were studied along with sex and age-matched controls attending the same general practice (Russell et al., 2005). In total, they matched 62 pairs of cases and controls, with a median age of disease onset in cases of 10.6 years. The study demonstrated that parental smoking during pregnancy and around the time of birth was more common in parents of IBD cases, 54% versus 29% in control parents (p=0.01; OR= 2.87, 95% CI= 1.23–6.66). Maternal smoking during pregnancy and at birth was also more common in IBD cases than in controls, 23% versus 6.2% (p=0.04; OR= 4.46, 95% CI= 1.16–17.1). Smoking in mothers of patients with CD was also greater, 27.8% versus 8.3% (p=0.03; OR= 4.23, 95% CI= 1.05–16.97). There was no significant effect seen when paternal smoking in pregnancy and at birth was analysed in IBD cases versus controls (p=0.27). This study was based on a very small sample, did not explore other ETS exposures and lacked control for potential confounding from SES. In a more recent study, Roberts et al. (2011) carried out a retrospective birth-cohort study in the UK. They used the Oxford record linkage study (ORLS) that comprised abstracts of records of birth registrations, maternities, day cases and inpatient admissions in the geographical region in and around Oxford. The maternity records covered a 20-year period from 1970 to 1989. These records were linked to all in-and-out patient visits until 1999. Diagnosis of IBD was based on requisite ICD codes for CD and UC. ETS exposure was "maternal smoking during pregnancy" as listed in the maternity records. Information on this exposure was available for 43 children with CD and 22 patients with UC. They observed increased risks of CD (OR=2.04, 95% CI=1.06-3.92, p=0.05) after accounting for potential confounding variables. A major strength of this study was that it was based within a well-defined geographic population, studied in a large cohort and included both inpatients and outpatients. Nonetheless, a major limitation was the unconfirmed IBD diagnosis that may have resulted in diagnostic misclassification. Furthermore, only 1 measure of ETS was assessed and the number of cases studied was small.

Table 2 - Childhood Passive Smoke Exposure and risk of adult-onset CD

Author, year of publication, location	Controls		Cases		OR (95% CI)
	Exposed	**Unexposed**	**Exposed**	**Unexposed**	
Persson et al., 1990, Stockholm	179	122	105	44	1.5 (1.0–2.3)
Thompson et al., 1995, United Kingdom	1013	476	1093	491	1.04 (0.88–1.23)
Eliakim et al., 2000, Israel	106	84	111	112	0.8 (0.5–1.1)
Feeney et al., 2002, United Kingdom	91	58	96	54	1.13 (0.69–1.86)
Bernstein et al., 2006, Manitoba	184	235	180	170	0.85 (0.61–1.20)
Mahid et al., 2007, Kentucky	183	59	217	36	2.04 (1.28–3.31)

Table 3. ETS exposure in childhood and risk of childhood-onset CD

Author, year of publication, location	Controls		Cases		OR (95% CI)
	Exposed	**Unexposed**	**Exposed**	**Unexposed**	
Lashner et al., 1993, Chicago	26	46	17	22	2.0 (0.5–8.0)
Rigas et al., 1993, New York	15	59	11	34	0.8 (0.3–2.5)
Baron et al., 2005, Northern France	156	66	144	78	0.84 (0.55–1.30)
Russell et al., 2005, Scotland	18	44	33	29	2.87 (1.23–6.66)
Roberts et al., 2011, South East England	34229	110934	16	27	1.92 (1.03-3.56)

MECHANISMS LINKING THE ASSOCIATION BETWEEN ETS/SMOKING AND CD

Several hypotheses have been proposed to explain the association between smoking and IBD, though none have convincingly demonstrated the reason behind the divergent effect of ETS on CD and UC. Nicotine was long believed to be the trigger; however, trials of nicotine replacement therapy in UC yielded equivocal results and no association was observed between oral tobacco use and CD (Lakatos et al, 2007, Cosnes et al, 2004). Smoking may influence the development of IBD through nicotinic acetylcholine receptors, which are present on mucosal epithelial cells of the bowel and on T cells. Clinical trials of nicotine replacement in UC have yielded modest yet inconsistent results; thus, nicotine alone may not be the sole component of smoking that influences IBD (Richardson et al, 2003, Razani-Boroujerdi et al, 2007, Birrenbach et al, 2006). Other proposed mechanisms are that the chemicals in smoke modulate cellular immunity, alter cytokine levels, modify colonic mucus production, and predispose to the development of microvascular thrombi or altered blood flow, suggesting that other components of tobacco smoke might be important (Frolkis et al, 2013). Smoking could alter smooth muscle tone and influence endothelial function through nitric oxide production, or affect the integrity of the gut mucous barrier (Hatoum et al, 2006, McGilligan et al, 2007). The effect of smoking could also be mediated by oxidative stress. Bergeron et al. (2012) found that mononuclear cells from smokers with CD, but not UC, were less sensitive to anti-inflammatory protection against oxidative free radical stress because of reduced levels of synthesis of heat shock protein. Polymorphisms in genes contributing to nicotine metabolism and cellular oxidative response might modify the susceptibility to smoke (Ananthakrishnan et al, 2014). Smoking also exerts an influence on the microbiota. Smoking cessation is associated with an early change in the microbiome, and this interaction with the immune response could underlie the effect of smoking cessation on UC (Biedermann et al, 2014, Munyaka et al, 2014, Parkes et al, 2014). Thus, a potential biological link between tobacco smoke and gut dysfunction does exist. Whether this link translates to increased risk for CD, however, remains unclear.

SUMMARY OF THE LITERATURE AND RATIONALE FOR CURRENT STUDY

CD has been recognized as a complex, multi-factorial disease. In spite of continuous research, no single environmental risk factor barring "active smoking" for adult CD, has been identified. Some studies in adults suggest that ETS exposure may also contribute to adult-onset CD. However, epidemiological findings for the association between ETS and child-hood onset CD have been inconsistent and limited. Most of the inconsistencies may be related to the "time period of exposure ascertained", potential recall bias, lack of control for potential confounders and sample size. Given the accumulating biological evidence, especially the chromosomal abnormalities in fetal epithelial cells of women who smoke during pregnancy (De la Chica et al, 2005), further investigation of this hypothesis is warranted. Thus, the main purpose of our study was to further clarify the role of ETS in childhood onset CD by examining different measures of ETS exposure.

OBJECTIVES

The major objectives of the study were:

1. To examine whether maternal and/or paternal smoking in the home during pregnancy was associated with risk of CD in children

2. To investigate whether post-natal exposure to maternal and/or paternal smoking was associated with risk of CD in children.

METHODS

STUDY DESIGN

A case-control study was conducted. The independent variable of interest was ETS exposure and the outcome (dependant variable), paediatric CD.

STUDY POPULATION

The study was based on an established cohort of patients with CD and controls recruited at Sainte-Justine Hospital, Montreal. At this study center, all patients (new or prevalent) diagnosed with CD since 2002 are included in an IBD registry. Clinical and socio-demographic information consisting of age at diagnosis, gender, modality used for diagnosis, maternal/paternal education, family history of IBD, treatments administered, etc. was abstracted from the medical records or acquired telephone interview. Children from different sources, including those visiting the orthopedic clinics of the hospital for minor fractures, and those visiting the gastroenterology units for diagnoses other than IBD, were recruited as controls to enhance population-representativeness. The selection of controls was restricted to those residing in the Greater Montreal Area. Information on baseline socio-demographic characteristics (age, gender, maternal education, family history of IBD, etc.) was collected. Various case-control studies examining the role of diet, hygiene and genetics (Amre et al, 2006, Amre et al, 2007, Springmann et al, 2014), etc. were carried out on subsets of this cohort. The sub-cohort selected for the current study is outlined below.

CASES

As the recruitment of cases is an ongoing process at the study hospital, only the most recently recruited patients were included. The study began in June 2013 and newly diagnosed patients were recruited as of 2012. The one-year gap was provided to allow for sufficient time to reach a confirmed CD diagnosis, as many cases need a change of diagnosis. As the disease is chronic, the majority of patients

were followed up over long periods of time at our tertiary care hospital. We then proceeded to include patients recruited during the previous years. In addition, we recruited patients that were enlisted in the IBD registry during the course of the current study (June 2013 to Jan 2015) and who had a confirmed diagnosis after a minimum one-year follow-up. The diagnosis of CD was established according to standard diagnostic procedures, including clinical data, endoscopy, radiology and histopathology (Baumgart et al, 2012). In the IBD literature, the term "paediatric" has many different definitions; upper age limits of 18, 20 and 21 years have all been previously used to designate paediatric-onset of the disease (Kim et al, 2004; Abraham et al, 2012; Bousvaros et al, 2006; Benchimol et al, 2011). In this study, the inclusion age was based on the age of the patients attending the gastroenterology clinic (0-20 years) of the study hospital. Cases with a diagnosis of UC or IC were excluded.

CONTROLS

As mentioned above, a cohort of non-IBD controls is continuously being established at the study centre (for investigating different hypotheses). Orthopaedic controls are children visiting the clinics for minor trauma (fracture) who do not have a history of IBD. Gastroenterology controls are children with a clinical suspicion of a gastrointestinal disorder that are found to be free of IBD following endoscopic evaluation. Therefore, included controls were diagnosed with either irritable bowel syndrome or intestinal polyps. No specific matching criteria were implemented, with the exception that controls were from the Greater Montreal Area. Area of residence was based on the first 3 digits of the postal code. For every case included in this study, we identified 1 control from the database who visited the clinic within 3 months of the case's visit. When more than control was available, we selected the one that visited the clinic at a date nearest to the date of diagnosis of the case. The selection of controls was based on the study-base principle, such that, if a control were to become a case, they would follow a path similar to that of the cases. As such, orthopaedic and gastrointestinal controls selected from the same hospital are expected to be from the same base population; and, if they had CD, they would likely be diagnosed at the study

hospital. ETS exposure has not been identified as a risk factor for either fractures, intestinal polyps or IBS and thus anticipating that inclusion of gastroenterology and/or orthopaedic controls would not result in selection bias.

STUDY PARTICIPATION & EXPOSURE INFORMATION (PLEASE SEE FIGURE IN APPENDIX 1)

The subjects identified for inclusion in the study were contacted and invited to participate in the collection of provisional of supplementary information (Note: most of the participants had already participated in previous investigations on IBD carried out by our team), specifically on ETS exposure. Cases and controls were concurrently invited to participate. For example, once a case agreed to participate, the corresponding potential control was contacted.

ENVIRONMENTAL TOBACCO SMOKE

In order ascertain exposure to ETS, mothers of the cases and controls were administered a structured questionnaire, specifically designed to ascertain ETS exposure, via telephone by trained interviewers (Appendix 2). The questionnaire covered various questions regarding ETS exposure during pregnancy, during the time period since birth and at the index date (date of diagnosis for cases and date at interview for controls). For exposure during pregnancy, mothers were asked whether they or their spouses smoked in the home. Smoking mothers were asked how often they smoked (never, sometimes, throughout), and how many cigarettes they smoked (1-5, 6-10, >10). For post-natal exposure, mothers were asked if the child had ever been exposed to ETS (from her or her partner/spouse smoking in the home) since birth and at the index date. Information on socio-demographic and clinical characteristics (such as age of diagnosis of the child, gender, family history of IBD in first-degree relatives, ethnicity, maternal and paternal education) had already been previously acquired from the participants either from the medical charts or via questionnaire.

POTENTIAL CONFOUNDERS

Based on the literature, we acquired information on potential confounders. These included characteristics such as age, and gender, wherein IBD is known to vary. As it is well known that the incidence of IBD varies according to race/ethnicity, and that the smoking characteristics of mothers vary according to ethnicity, we acquired information on the ethnic origins of the child. Some studies have suggested that socio-economic status (SES) may be related to IBD, wherein IBD is more common in those within the higher socio-economic strata. We acquired information on maternal and paternal education as markers of SES. Finally, family history is strongly associated with CD. We thus acquired detailed information on the presence of IBD in the first degree relatives of the subjects.

ETHICAL CONSIDERATIONS

The study was approved by the Ethics Committee of *Centre Hospitalier Universitaire Sainte-Justine,* and informed consent was acquired from all participants.

STATISTICAL ANALYSIS

STATISTICAL PROGRAM

The statistical program used for the analysis was Stata (version 10.1).

Variables in the dataset

Participant characteristics	**ETS variables**
ID*	ETS exposure in the home during pregnancy and the smoking party (mother or father or both)
Date of birth	Frequency of maternal smoking during pregnancy
Age at diagnosis for cases and recruitment for controls	
Gender	Number of cigarettes smoked by the mother during pregnancy
Area of residence	
Maternal education	
Date of diagnosis/Date of interview	
Case-control**	

---

*Unique identifier for each participant

**This dichotomous variable indicated whether the participant was a case or a control

DESCRIPTIVE ANALYSIS

The goal of this analysis was to assess the distribution of the characteristics of the cases and controls to identify missing values, errors and potential outliers, if any. For continuous variables, summary statistics such as mean (± SD) and range were estimated to identify observations not lying within the expected distribution. For categorical variables, the proportions within the categories were assessed.

The initial analysis was univariate, wherein, the distribution of the selected characteristics was compared between the cases and the controls. T-tests were used for continuous variables and chi-square or fisher's exact tests for categorical variables. Statistical significance was set at $p<0.05$.

DEFINITION OF ETS EXPOSURES

Exposure to ETS in the home during pregnancy:

The most common ETS exposure to the fetus is via passive smoke exposure acquired directly via maternal smoking and via exposure from other individuals (usually the father/common law spouse/partner) who smoke around the pregnant mother. As this exposure is most intense in closed environments in the majority of cases in the home, we assessed:

1. Whether the mother smoked in the home when pregnant (yes/no)
2. Whether the father smoked in the home when the mother was pregnant (yes/no)

Exposure to ETS in the home post pregnancy:

In order to measure the impact of post-natal ETS exposure and its links with CD, ETS exposure in the home was assessed for mothers and fathers.

Strategy for detecting ETS exposure effects

It is well known that assessing the independent effects of pre-natal and post-natal ETS effects on health outcomes can be challenging. In order to determine the potential effects and detect residual confounding Yang et al (2013) outlined a comprehensive strategy. They suggested:

For prenatal effects:

1. To examine the association between maternal smoking during pregnancy in two ways:
    a. Compare associations between children whose mothers smoked during pregnancy with those children whose mothers never smoked (either during pregnancy or subsequently)
    b. Compare associations between children whose mothers smoked both during and after pregnancy with those children whose mothers smoked only after pregnancy (and not during)

After adjusting for paternal smoking (during and after pregnancy), associations, if any, should be present for both a) and b) comparisons. In the absence of consistency and in the presence of stronger associations with paternal smoking (as compared to maternal smoking), it can be concluded that associations, if any, with maternal smoking were likely due to residual confounding.

For post-natal effects:

Comparisons can be made between children whose mothers did not smoke during pregnancy, but only after pregnancy, with those children whose mothers smoked at neither period. After adjusting for paternal smoking (both during and after pregnancy), if real associations are present, they should manifest with both maternal and paternal smoking; but, those for maternal smoking would be expected to be of larger magnitude as children tend to spend more time with their mothers.

Based on the above recommendations we adopted the following strategy for our study.

Prenatal ETS exposure in the home

1. We compared children of mothers who smoked during pregnancy with children of mothers who smoked neither during nor after pregnancy.
2. We compared children of mothers who smoked during or after pregnancy with children of mothers who smoked only after pregnancy.
3. We compared children whose fathers smoked during pregnancy versus those who did not, among mothers who never smoked (neither during pregnancy nor afterwards).

Post-natal ETS exposure

1. We compared children of mothers who smoked ONLY after pregnancy versus children of mothers who never smoked.
2. We compared children whose fathers smoked post-pregnancy with children whose fathers did not smoke post-pregnancy, among mothers who never smoked.
3. We compared children whose fathers smoked both during pregnancy and post-pregnancy with children whose fathers never smoked, among mothers who never smoked.

We fit 2 models:

1. Crude model, not accounting for any confounding variables, and
2. Adjusted model, adjusting for covariates that were significantly associated with case-control status in univariate analysis. In addition, when examining associations with pre-natal paternal smoking, we adjusted for post-natal paternal smoking and vice versa.

Linearity assumptions, model specification and fit, and the presence and effect of outliers/influential observations were checked using standard post-estimation methods (Appendix 3). Odds ratios (OR) and 95% confidence intervals (95% CI) were estimated.

SAMPLE SIZE/POWER

A sufficiently large sample size ensures that the risk of making a type II error is acceptable (Rossiter et al, 1982). Type II errors occur when the null hypothesis (in this case, that ETS exposures are not associated with CD) is accepted when it should be rejected. We *a priori* estimated the required sample size for our study. Our goal was to have sufficient power (80%) to detect meaningful associations (Odds ratios >2) at an alpha level of 0.05 for a case-control ratio of 1:1. For anticipated ETS exposure we used the prevalence of maternal smoking during pregnancy as a proxy. For the Quebec population this frequency ranged from 20% to 30% during 1993 to 2007 (PHAC, 2013). As this time period corresponded with the birth period of our case-control sample, we estimated sample sizes considering this range of potential ETS exposures. The results are presented in Appendix 4. We estimated that we would require between 85 to 170 case-control pairs to detect an OR between 2.0 to 2.5 for ETS exposures ranging from 20% to 30%. The Quanto program was used to carry out the power calculations (Gauderman, 2006).

RESULTS

The results of the study are presented in article format.

The following manuscript, entitled “Environmental Tobacco Smoke exposure and risk of Crohn’s disease in children” **will be submitted** to the Journal “Tobacco Control”. The following is a list of the co-authors and their contributions, in the order in which they appear on the manuscript:

- Ali Lalavi: methodology, data cleaning, statistical analysis, writing of the manuscript
- Carl-Duchatellier : data collection
- Devendra Amre: study design, methodology, custodian of the database, statistical analysis, manuscript edits

INTRODUCTION

The incidence of childhood-onset Crohn's disease (CD), a recurrent, relapsing inflammatory disease of the gastro-intestinal tract, is among the highest in Canada (Fedorak et al, 2010). Although much progress has been made in understanding the etiopathogenesis of the disease, the critical triggers that activate and maintain the aberrant inflammatory response remain unclear. Much attention has been focused on clarifying potential environmental triggers. One exposure of interest is exposure to environmental tobacco smoke (ETS). In adults, active smoking predisposes individuals to CD and is protective for ulcerative colitis (UC) (Ng et al, 2013). The evidence for whether passive smoke exposure predisposes patients to adult-onset CD remains unclear (Jones et al, 2008). Similar efforts to investigate whether ETS exposure could enhance the susceptibility for childhood-onset CD were met with limited success, with some studies suggesting increased risks (Lashner et al, 1993; Russel et al, 2005; Roberts et al, 2011) and others suggesting none (Rigas et al, 1993; Baron et al, 2005). A combination of factors, such as limited power, targeting different exposure windows, and not controlling for potential residual confounding could be responsible for the heterogeneous findings. Mechanistic studies do seem to indicate that constituents of cigarette smoke could alter the gut mucosa (Lakatos et al, 2007; Hatoum et al, 2006; McGilligan et al, 2007) and/or the gut microflora (Munyaka et al, 2014; Parkes et al, 2014), and thereby alter immune homeostasis. These mechanisms seem to explain the known associations between ETS exposure and adult-onset CD, but whether they can predispose children to CD is yet to be determined. The main purpose of this study was thus to further clarify the role of ETS exposure during critical time periods (during pregnancy and post-pregnancy) and risk of CD in children.

METHODS

We carried out a retrospective case-control study in children diagnosed with CD prior to age 20 recruited from the gastrointestinal clinics of Sainte-Justine Hospital in Montreal. CD was diagnosed using standard criteria. Controls were children without a diagnosis of CD from outpatient clinics in the orthopaedic departments treated for minor trauma and children visiting the gastroenterology clinics for abdominal symptoms, but in who IBD was ruled out after endoscopy. No specific matching criteria were implemented, with the exception that cases and controls were restricted to those residing in the Greater Montreal Area (based on the first 3 digits of the postal code).

The study sample was a sub-cohort of an established cohort of patients and controls. Since 2002, at the study centre, patients newly diagnosed with CD with a diagnosis confirmed after a minimal follow-up of one year, and controls from the orthopaedic and gastroenterology clinics are included into a IBD registry. Research on this cohort has been previously carried out (Amre et al, 2006; Amre et al, 2007). The current study began in June 2013 and concluded in January 2015. We first included patients and controls diagnosed in 2012. For each patient, we selected a control from the registry that was diagnosed within 3 months of the corresponding case and resided in the Greater Montreal Area. We contacted and invited the subjects for participation in the current ETS study. We then proceeded to recruit subjects from the previous years. We also included subjects that were newly registered in the registry during the study period. We concluded the recruitment of subjects for this study once the required number was met based on *a priori* sample size estimations (please see below).

To acquire information on ETS exposure, the mothers of the subjects were administered a structured questionnaire over telephone by trained interviewers. The questionnaire covered various questions regarding ETS exposure during pregnancy and during the time interval from birth to the index date (date of diagnosis for cases and date of interview for controls). For exposure during pregnancy, mothers were asked whether they or the child's father smoked in the home during the pregnancy. Smoking mothers were asked how many cigarettes they consumed (1-5, 6-10, >10). For post-natal ETS

exposure, mothers were asked if they or the father smoked in the home from the birth of the child until the index date. Information on socio-demographic and clinical characteristics (such as age of diagnosis of the child, gender, family history of IBD in first-degree relatives, ethnicity, maternal and paternal education) was abstracted from the previously established IBD database. This information had been acquired from the medical charts or via a supplementary questionnaires.

DEFINITION OF ETS EXPOSURES

Exposure to ETS in the home during pregnancy:

The most common ETS exposure to the fetus is from passive smoke exposure acquired directly via maternal smoking and via exposure from other individuals (usually the father) who smoke around the pregnant mother. As this exposure is most intense in closed environments, in majority of the cases in the home, we assessed:

1. Whether the mother and/or the father smoked in the home (categorical variable: 0=neither parent smoked; 1=only the mother smoked; 2=only the father smoked; 3=both parents smoked)
2. A composite measure of household ETS exposure wherein the exposure was either via maternal smoking or paternal smoking (binary)

Exposure to ETS in the home post pregnancy:

In order to measure the impact of post-natal ETS exposure and its links with CD, similar to household ETS exposure during pregnancy, an ETS exposure measure in the home was assessed for mothers and fathers.

Sample size and power

We *a priori* estimated the required sample size for our study. Expected ETS exposure in our sample was based on reports of the prevalence of maternal smoking during pregnancy among women in Quebec (PHAC, 2013). This report provided estimates on the prevalence of ETS from 1993-2007, a period that corresponded with the birth periods of our study population. We used these estimates (20% to 30%), to calculate the required sample size for detecting meaningful associations (Odds ratios >2) at an alpha level of 0.05, for a case-control ratio of 1:1, with adequate power (80%). We estimated that we would require between 85 to 170 case-control pairs to detect an OR between 2.0 to 2.5. The Quanto program was used to carry out the power calculations (Gauderman, 2006).

STATISTICAL ANALYSIS

We adapted the suggestions of Yang et al., (2013) to detect associations between ETS resulting from either maternal or paternal smoking at the two time periods (prenatal and postnatal).

Prenatal ETS exposure in the home

1. We compared children of mothers who smoked during pregnancy with children of mothers who smoked neither during nor after pregnancy.
2. We compared children of mothers who smoked during or after pregnancy with children of mothers who smoked only after pregnancy
3. We compared children whose fathers smoked during pregnancy versus those who did not, among mothers who never smoked (neither during pregnancy nor afterwards)

Post-natal ETS exposure

1. We compared children of mothers who smoked ONLY after pregnancy versus children of mothers who never smoked

2. We compared children whose fathers smoked post-pregnancy with children whose fathers did not smoke post-pregnancy, among mothers who never smoked
3. We compared children whose fathers smoked both during pregnancy and post-pregnancy with children whose fathers never smoked, among mothers who never smoked.

We fit 2 models:

1. Crude model, not accounting for any confounding variables, and
2. Adjusted model, adjusting for covariates that were significantly associated with the case-control status in univariate analysis. In addition, when examining associations with pre-natal paternal smoking, we adjusted for post-natal paternal smoking and vice versa.

Linearity assumptions, model specification and fit, and the presence and effect of outliers/influential observations were checked using standard post-estimation methods. Odds ratios (OR) and 95% confidence intervals (95% CI) were estimated.

RESULTS

There were approximately 500 newly-diagnosed CD patients and an equal of number of controls available for selection in the database. Among the patients approached, 132 cases (152 contacted, 86%response rate) and 132 controls (160 contacted, 82%response rate) (Table 1) participated in the study. For 1 control, the data acquired contained numerous missing entries on key variables. After attempts to re-contact failed, the control was excluded from the study. The corresponding case was, however, retained to make full use of the available data. The mean (±SD) age of the cases: 12.1 (±3.6) was slightly higher than the controls: 11.4 (±4.7). There were no gender differences between the cases and controls. Family history of IBD was more prevalent among the cases. Ethnicity, maternal education and paternal education were similarly distributed among the comparison groups. Participating and non-participating subjects did not differ with regards to the above characteristics (data not shown).

Crude analysis (Table 2) showed that, with regards to prenatal ETS, maternal smoking during pregnancy was not associated with risk of CD in children. There was some evidence that paternal exposure was positively associated with risk of CD (OR=1.93, 95% CI=0.99-3.75, p-value=0.054). With regards to post-natal ETS exposure, the results suggested that children whose mothers smoked only after pregnancy were more likely to be at risk for CD (OR=3.91, 95% CI=0.79-19.3, p=0.09) compared to children whose mothers never smoked. When paternal smoking, among mothers who never smoked was evaluated, children who were exposed to post-natal paternal smoking were more likely to be at risk for CD (OR=2.62, 95% CI=1.19-5.74, p-value=0.02) in comparison with children whose fathers did not smoke. Results were similar when paternal smoking, pre-and post-pregnancy was evaluated. Children whose fathers who smoked during both time periods were more likely to be at risk for CD (OR=2.53, 95% CI=1.14-5.63, p-value=0.02) in comparison with children whose fathers never smoked. The latter analysis was carried out among non-smoking mothers.

In the univariate analysis (Table 1), only age and family history of IBD were associated with the case-control status. Given the small subset of patients for most analyses, it was deemed appropriate to fit a parsimonious multivariate model that only adjusted for these two potential confounding variables. Results for the adjusted analysis (Table 3) mirrored that of the crude analysis. There was no evidence of risk associated with exposure to ETS during pregnancy. After adjusting for paternal post-natal smoking, associations with pre-natal paternal smoking were no longer evident (among mothers who never smoked) (OR=0.95, 95% CI=0.33-2.75, p-value=0.93). ETS exposure post-pregnancy remained positively associated with CD. Children whose mothers smoked only after pregnancy appeared to be at increased risk for CD, albeit, these associations were not statistically significant (OR=3.54, 95% CI=0.71-17.57, p-value=0.12). However, after additional adjustment for pre-natal paternal smoking, post-natal paternal smoking appeared to confer a higher risk of CD in children (OR=2.75, 95% CI=0.81-9.29, p-value= 0.10). Children exposed to ETS via their fathers' smoking, both during pregnancy and after pregnancy, were susceptible to similarly higher risks of CD.

Overall the results suggested that:

1. There was NO association between ETS exposure in the home during pregnancy and risk of CD in the child
2. Paternal smoking during pregnancy was NOT associated with higher risks of CD
3. There appeared to be positive associations between post-natal ETS exposure in the home and future risk of CD in the child both via maternal and paternal smoking.

DISCUSSION

Environmental risk factors have long been touted to contribute to the occurrence of CD in children. Findings of genome-wide association studies (GWAS), indicating that genetic factors explain less than 25% of the variability in CD, further support the role for environmental risk factors in the etiology of CD (Jostins et al, 2012). However, research thus far has not identified any specific environmental risk factor. Many risk factors, such as high dietary consumption of fats, low consumption of fruits and vegetables (Amre et al, 2006), and low exposure to infections (Springmann et al, 2014) have been implicated in CD development. Nevertheless, the exact role of these exposures is yet to be established.

The role of environmental risk factors remains elusive for both childhood and adult-onset CD. In adult-onset CD, however, smoking is a known risk factor (Ng et al, 2013). A number of studies tried to explore whether the same is true for childhood-onset CD. Given that active smoking is unlikely to play a role, the focus has been on passive exposure to tobacco smoke. Towards this end, the role of tobacco smoke exposure (ETS) during pregnancy and post-pregnancy, in particular, has garnered attention. Current studies, however, have not reached any conclusions, as shown in the meta-analysis by Jones et al, (2008). The investigation of different time periods, potential for recall bias, and low power may have

hampered the ability to ascertain definitive conclusions. Thus, the role of ETS exposure in childhood-onset CD remains unclear.

Numerous lines of investigation support the potential link between tobacco smoke exposure and inflammation (Verschuere et al, 2012). Tobacco smoke leads to intestinal muscle dysfunction (Hatoum et al, 2006; McGilligan et al, 2007), increases the production of oxidative stress (Bergeron et al., 2012), alters the gut microflora (Munyaka et al, 2014; Parkes et al, 2014) and increases the production of pro-inflammatory cytokines (Frolkis et al, 2013). These changes may be more prominent with active smoke exposure and may underlie the strong and consistent associations reported with adult-onset CD. It is possible that such exposures, acquired via passive smoke exposure, especially during the critical phases of development, such as in utero, could confer similar risks for childhood-onset CD. Although passive exposure is expected to confer lower exposures to smoke as compared to active smoking, even low dose exposure may confer risk given the critical developmental stage. Our findings of an absence of increased risks from ETS exposure during pregnancy may suggest that low dose exposure to chemicals in tobacco smoke may be insufficient in conferring CD risk. Our findings are consistent with previous research. For example, in a case-control study of 68 patients with CD and 202 controls in New York, Rigas et al, (1993) did not find any associations between maternal smoking during childhood and risk of CD in childhood. Similarly, Baron et al, (2005) did not find associations between maternal smoking during pregnancy and risk of CD in childhood. Our results, however, differ from the findings of Lashner et al, (1993), Russell et al, (2005) and Roberts et al, (2011). These studies found an association between ETS exposure during pregnancy or at birth and risk of CD. The reasons for these discrepancies could be manifold. The Lashner et al, (1993) study (CD=39 cases, 72 controls) and the Russell et al, (2005) (62 matched case-control pairs) studies were based on small samples, and the resulting findings could have been overestimated. Lashner et al, (1993) did not evaluate ETS exposure during pregnancy (but at birth), and did not account for potential confounding variables such as SES and family history of IBD. Similarly, Russell et al, (2005) did not adjust for potential confounding variables. The Roberts et al, (2011) study

was a retrospective cohort study based on administrative data and had a sample size equivalent to that of our study (114 cases, 248479 controls). It, however, relied on record linkage and the diagnosis of CD was not confirmed. Furthermore, the data on smoking was not actively collected, but extracted from inpatient/outpatient files and missing data was another limitation.

With regards to post-natal smoking, only one study has investigated the associations with childhood-CD (Baron et al, 2005). They reported no associations. The reasons for the discrepancy with our study are unclear. The prevalence of passive smoke exposure during childhood in the controls was much lower in the Baron et al, (2005) study (14%) as compared to our study (42.9%), suggesting that exposure ascertainment may have been incomplete in their study. Lack of information on the exact time window they investigated also hampers adequate comparisons between these two studies.

Our study has inherent limitations given its retrospective nature. Exposure to ETS was based on the recollection of mothers' smoking history, which at times dated back 20 years. Thus, recall bias is a limitation of our study. It is well known that mothers under-report their smoking exposures during pregnancy (Dukic et al, 2009). Whether such underreporting, if any, was differential with regards to the case-control status in our study is an important concern. The observed lack of associations with maternal exposure during pregnancy and CD may suggest that case mothers may be selectively underreporting their exposures more than control mothers. Although we collected information on the number of cigarettes smoked, we did not investigate a dose-response relationship of ETS as quantifying its exposure is likely more susceptible to recall bias than reporting ETS exposure as yes/no.

Our comprehensive analysis suggested that studies examining the effects of ETS exposure during different time periods need to critically examine the results to identify the potential presence of residual confounding. Towards this end, suggestions of positive associations between post-natal ETS exposure (either maternal or paternal) and CD could reflect the presence of residual confounding. Families that smoke differ substantially from those that do not (in terms of SES, attitudes towards children, beliefs, coping mechanisms, etc.) (Cnattingius et al, 1992; Fingerhut et al, 1990; Glassman et al, 1990; Kahn et al,

2002; Smedberg et al, 2014). Thus, potential confounding related to behaviour (stress for example) may account for the observed positive associations. More direct measures of ETS exposure (such as nicotinine levels in the parents and children) carried out in prospective cohorts may provide better information on the link between ETS exposure during the pre-natal and post-natal period and the risk of CD in childhood.

In conclusion, our study suggests that passive exposure via maternal smoking *per se* during pregnancy is not associated with increased risks of CD in children. Post-natal ETS exposure, however, either via paternal or maternal smoking appears to enhance risks. Given the narrow time window for exposures during pregnancy and the potentially longer duration and intensity of post-natal ETS exposures, these findings are relevant. Nonetheless, additional studies are required to further examine these associations.

REFERENCES

Amre, DK., D'Souza S, Morgan K, et al. Imbalances in dietary consumption of fatty acids, vegetables, and fruits are associated with risk for Crohn's disease in children. Am J Gastroenterol 2007;102(9):2016-2025.

Amre, DK, Lambrette P, Law L, et al. Investigating the Hygiene Hypothesis as a Risk Factor in Pediatric Onset Crohn's Disease: A Case-Control Study. Am J Gastroenterol 2006;101(5):1005-1011.

Springmann V, Brassard P, Krupoves A, et al. Timing, frequency and type of physician-diagnosed infections in childhood and risk for Crohn's disease in children and young adults. Inflamm Bowel Dis 2014;20(8):1346-1352.

Baron S, Turck D, Leplat C, et al. Environmental risk factors in paediatric inflammatory bowel diseases: a population based case control study. Gut 2005;54(3): 357-363.

Bergeron, V, Grondin V, Rajca S, et al. Tobacco and IBD: relevance in the understanding of disease mechanisms and clinical practice. Best Pract Res Clin Gastroenterol 2004;18(3):481-496.

Cnattingius S, Lindmark G, Meirik O. Who continues to smoke while pregnant? J Epidemiol Community Health 1992;46(3):218-221.

Dukic VM, Niessner M, Pickett KE, et al. Calibrating self-reported measures of maternal smoking in pregnancy via bioassays using a Monte Carlo approach. Int J Environ Res Public Health 2009;6(6):1744-1759.

Dupont-Lucas C, Amre DK. Diet-gene interactions in the pathogenesis of Crohn's disease : The polyunsaturated fatty acids (PUFA) metabolic pathway as a prototype. Curr Nutr Reports 2015;4:201-2088.

Fedorak, RN, Wong K, Bridges R. Canadian Digestive Health Foundation Public Impact Series. Inflammatory bowel disease in Canada: Incidence, prevalence, and direct and indirect economic impact. Can J Gastroenterol 2010;24(11):651-655.

Fingerhut LA, Kleinman JC, Kendrick JS. Smoking before, during, and after pregnancy. Am J Public Health 1990;80(5):541-544.

Frolkis A, Dieleman A, Barkema HW, et al. Risk of surgery for inflammatory bowel diseases has decreased over time: a systematic review and meta-analyses of population-based studies. Gastroenterol 2013;145(5):996-1006.

Gauderman W, Morrison J. A computer program for power and sample size calculations for genetic-epidemiology studies. QUANTO In. 1.1 ed; 2006

Glassman AH, Helzer JE, Covey LS, et al. Smoking, smoking cessation, and major depression. JAMA 1990;264(12):1546-1549.

Hatoum, OA, Heidemann J, Binion DG. The Intestinal Microvasculature as a therapeutic target in inflammatory bowel disease. Ann N Y Acad Sci 2006;1072:78-97.

Jones DT, Osterman MT, Bewtra M, et al. Passive Smoking and Inflammatory Bowel Disease: A Meta-Analysis. Am J Gastroenterol 2008;103(9):2382-2393.

Jostins L, Ripke S, Weersma RK, et al. Host–microbe interactions have shaped the genetic architecture of inflammatory bowel disease. Nature 2012;491(7422):119-124.

Kahn RS, Certain L, Whitaker RC. A reexamination of smoking before, during and after pregnancy. Am J Public Health 2002;92(11):1801-1808.

Lakatos PL. Smoking in inflammatory bowel diseases: Good, bad or ugly? World J Gastroenterol 2007;13(46):6134.

Lashner BA, Shaheen NJ, Hanauer SB, et al. Passive smoking is associated with an increased risk of developing inflammatory bowel disease in children. Am J Gastroenterol 1993;88(3):356-359.

McGilligan VE, Wallace JMW, Heavey PM, et al. Hypothesis about mechanisms through which nicotine might exert its effect on the interdependence of inflammation and gut barrier function in ulcerative colitis. Inflammatory Bowel Diseases 2007;13(1):108-115.

Munyaka PM, Khafipour E, Ghia JE. External Influence of Early Childhood Establishment of Gut Microbiota and Subsequent Health Implications. Front Pediatr 2014;9:2.

Ng SC, Bernstein CN, Vatn MH, et al. Geographical variability and environmental risk factors in inflammatory bowel disease. Gut 2013;62(4):630-649.

Parkes GC, Whelan K, Lindsay JO. Smoking in inflammatory bowel disease: Impact on disease course and insights into the etiology of its effect. J Crohn's Colitis 2013;8(8):717-725.

Rigas A, Rigas B, Glassman M, et al. Breast-feeding and maternal smoking in the etiology of Crohn's disease and ulcerative colitis in childhood. Ann Epidemiol 1993;3(4):387-392.

Roberts SE, Wotton CJ, Williams JG, et al. Perinatal and early life risk factors for inflammatory bowel disease. World Journal of Gastroenterology 2011;17(6):743-749.

Russell RK, Farhadi R, Wilson M, et al. Perinatal passive smoke exposure may be more important than childhood exposure in the risk of developing childhood IBD. Gut 2005;54(10): 1500-1501.

Smedberg J, Lupattelli A, Mardby A-C, et al. Characteristics of women who smoke during pregnancy: a cross-sectional study of pregnant women and new mothers in 15 different European countries. BMC Pregnancy Childbirth 2014;14:213.

Verschuere S, De Smet R, Allais L, et al. The effect of smoking on intestinal inflammation: what can be learned from animal models? J Crohns Colitis 2012;6(1):1-12.

Yang S, Decker A, Kramer MS. Exposure to parental smoking and child growth and development. A cohort study. BMC Ped 2013;13:104.

Table 1: Characteristics of the study population

Characteristic	Controls N=131	Cases N=132	P-value
Age*			
(Mean ±SD)	11.4 (4.7)	12.7 (4.0)	0.01**
Gender			
Male	67 (51.2)	70 (53.0)	
Female	64 (48.8)	62 (47.0)	0.76
Family history of IBD			
No	111 (86.7)	98 (74.8)	
Yes	17 (13.3)	33 (25.2)	0.015**
Ethnicity			
Non-Caucasian	4 (3.0)	7 (5.3)	
Caucasian	127 (96.9)	125 (94.7)	0.36
Maternal education			
High school	38 (29.9)	53 (42.4)	
College	46 (36.2)	42 (33.6)	
University	43 (33.9)	30 (24.0)	0.08
Paternal education			
High school	50 (38.8)	60 (46.1)	
College	30 (23.3)	32 (24.6)	
University	49 (38.0)	38 (29.2)	0.31

* Age at diagnosis for cases and at recruitment for controls ** P-value significant

Table 2: Association between environmental tobacco smoke exposure during and after pregnancy and risk of Crohn's disease in children. Crude analysis.

Exposure	Controls	Cases	OR (95% CI)	P-value
Exposure during pregnancy				
Smoking in the home				
Maternal smoking during pregnancy versus never smoking				
No	104 (52.8)	93 (74.4)		
Yes	25 (47.2)	32 (25.6)	1.43 (0.79-2.59)	0.24
Maternal smoking during and post-pregnancy versus only post-pregnancy smoking				
No	2 (22.2)	7 (18.9)		
Yes	17 (77.8)	30 (81.1)	0.50 (0.09-2.71)	0.42
Paternal smoking during pregnancy among never-smoking mothers				
No	85 (56.7)	65 (69.9)		
Yes	19 (43.3)	28 (30.1)	1.93 (0.99-3.75)	0.054
Post-natal exposure				
Maternal postnatal smoking only versus never smoking				
No	104 (52.8)	93 (93.0)	3.91 (0.79-19.3)	0.09
Yes	2 (47.2)	7 (7.0)		
Paternal smoking post-pregnancy among never smoking mothers				
No	93 (56.7)	71 (76.3)		
Yes	11 (43.3)	22 (23.7)	2.62 (1.19-5.74)	0.02
Paternal smoking pre- and post-pregnancy versus never smoking among never smoking mothers				
No	85 (57.1)	64 (75.3)		
Yes	11 (42.9)	21 (24.7)	2.53 (1.14-5.63)	0.02

* P-value significant

Table 3: Association between environmental tobacco smoke exposure during and after pregnancy and risk of Crohn's disease in children. Multivariate analysis*

Exposure	OR (95% CI)	P-value
ETS during pregnancy		
Maternal smoking during pregnancy versus never smoking	1.55 (0.84-2.86)	0.16
Maternal smoking during and post-pregnancy versus only post-pregnancy smoking	1.47 (0.80-2.71)	0.21
Paternal smoking during pregnancy versus not smoking among never-smoking mothers†	0.95 (0.33-2.75)	0.93
Post-natal exposure		
Maternal postnatal smoking only versus never smoking††	3.54 (0.71-17.57)	0.12
Paternal smoking post-pregnancy versus not smoking, among never smoking mothers†	2.75 (0.81-9.29)	0.10
Paternal smoking pre- and post-pregnancy versus never smoking among never smoking mothers	2.52 (1.11-5.72)	0.03**

* All models were adjusted for age at diagnosis, and family history of IBD

† Adjusted for prenatal paternal smoking or postnatal smoking

†† Adjusted for paternal smoking during pregnancy & paternal smoking after pregnancy

** P-value significant

DISCUSSION

It is now widely recognized that ETS exposure is associated with a wide-range of health effects. Although much of the focus has been on its links with respiratory effects, emerging evidence suggests that ETS exposure can increase the risk of developing many acute and chronic illnesses (Seong et al, 2015). The biological effects of ETS exposure, especially those that occur during pregnancy, are well documented. A major effect of ETS is on the anti-oxidant system in the placenta, which gets perturbed due to the exposure to toxins, leading to increased lipid peroxidation. The resulting increase in free radicals can perturb embryogenesis at different stages leading to both short-term and long-term health consequences (Dittrich et al, 2012; Chelchowska et al, 2011; Sahinli et al, 2012).

CD is a chronic, recurring, inflammatory disease of the gastro-intestinal tract. This complex disorder is thought to result from an interaction between multiple factors that include environmental, genetic, and immune factors. Much is now known about the contribution of genetic factors with approximately 150 variants being identified (Jostins et al, 2012). Long-standing research on potential environmental risk factors has in comparison been less successful. Much of the latter could be attributed to the difficulty in both identifying and measuring such risk factors, the low incidence of disease and the indolent pathogenesis. Not barring these limitations, the single most consistent risk factor in adults is cigarette smoking that curiously is associated with increased risk of CD and decreased risk of UC, the other form of IBD. The mechanisms leading to increased or decreased risks, however, remain unclear (Ananthakrishnan, 2015).

CD in children remains a major clinical dilemma. The heterogeneous nature of the disease, associated complications and social and psychological effects make its management extremely challenging. Why some individuals are more susceptible to disease early in life and why others only acquire disease in adulthood is a topic of intense investigation. Genetic differences were considered. However, it is now clear that both childhood-onset and adult-onset CD share genetic similarities (Jostins et al, 2012). Thus, variability in environmental exposures such as a predisposition has received much attention. Towards this end, imbalances in dietary exposures (Amre et al, 2006), and exposure to

infections (Amre et al, 2007; Springmann et al, 2014) have been documented; but no specific agents have been consistently identified. Given the known association between active smoking and CD, determining whether ETS exposure, either during pregnancy or post-natal, can increase the risk of childhood-onset CD, has also received some attention. However, findings from the 5 studies carried out thus far have been inconsistent. Differences in defining ETS exposure, ETS measurements, recall bias, controlling for potential confounding factors and/or limited power may be the underlying cause of these inconsistencies. Therefore, we conducted the current study to further explore the association between ETS exposure and risk for childhood-onset CD.

Our case-control study, based on newly-diagnosed patients and hospital-based controls, suggests that ETS exposure during pregnancy is not associated with risk of CD in children. In contrast, post-natal ETS exposure via either maternal or paternal smoking in the home appeared to have contributed to increased risk of CD. Though these findings are of interest, they need to be interpreted keeping in mind the potential study limitations described below.

1. The greatest difficulty in attempting to examine the association between ETS exposure and a chronic disease such as CD is the ability to accurately measure ETS. As CD is a rare disease, and even rarer in children, designing a prospective study is not feasible. This precludes prospective measures of ETS by biomarkers or questionnaires. We therefore implemented a case-control design. This design requires the use of questionnaires to ascertain past exposure to ETS. Recall bias is a major concern for this type of study design. We attempted to reduce its impact by including newly diagnosed patients and controls in order to limit the time from exposure to disease onset and study start. Nonetheless, for ETS exposure during pregnancy, recall bias remained challenging, particularly for children diagnosed later in childhood.

   Another related challenge was the potential mixing of ETS exposures. The mother provided information on ETS exposure. Accurate quantification of ETS is impossible in the absence of comprehensive information on the number of cigarettes smoked, the duration of smoking, whether smoking was mostly outdoors or indoors, and the quality of indoor ventilation.

Though the frequency of maternal smoking during pregnancy we observed (~20%) is equivalent to that reported among Quebec women (PHAC, 2013), noted associations with prenatal ETS need to be interpreted with caution.

Another challenge was ascertaining post-natal exposure prior to disease. The mother was asked whether the child had been exposed to ETS since birth prior to CD diagnosis or recruitment. It was not possible to further characterize the timing or duration of this exposure. Moreover, information regarding this exposure did not include potential exposures to active smoking, especially by adolescents and young adults. Thus, caution is again required in interpreting the related findings.

2. The lack of associations with prenatal ETS exposure we reported could have been the result of differential misclassification. It is well known that mothers underreport their smoking during pregnancy (Dukic et al, 2009). With increasing access to information, in particular with regards to the association between smoking and adult-onset CD, mothers of children with CD may have underreported their ETS exposure more than mothers of children without CD. On the other hand, our study was powered for detecting effects greater than 2.0. More modest associations could thus have been missed. Larger studies will be needed.
3. We reported increased risks of CD from ETS exposure for parents who smoked both during pregnancy and during childhood. It was impossible to determine the exact contribution of paternal smoking during pregnancy and after pregnancy. The latter is due to our analysis of the subgroup of fathers who “did not smoke during pregnancy” and only smoked “after pregnancy”, among mothers who never smoked (there was only 1 father). Analysis of this sub-group is important in order to estimate the independent effects.
4. The effects of ETS exposure, if any, are likely mediated by interactions with other exposures such as diet and the gut microflora. We did not have information on these factors for many of our study subjects, thus precluding the investigation of potential interactions.

COMPARISON WITH PREVIOUS STUDIES

Five previous studies have examined the association between ETS exposure and risk of CD in children. Four of these studies (Lashner et al, 1993; Rigas et al, 1993; Baron et al, 2005 and Russell et al, 2005) were case-control studies, whereas the study by Roberts et al, (2011) was a retrospective cohort study. Our findings are consistent with those of previous studies. For example, in a case-control study carried out in 68 patients with CD and 202 controls in New York, Rigas et al, (1993) did not find any associations between maternal smoking during childhood and risk of CD in childhood. Similarly, Baron et al, (2005) did not find any associations between maternal smoking during pregnancy and risk of CD in childhood. Our findings, however, differ from those of Lashner et al, (1993), Russell et al, (2005) and Roberts et al, (2011), which found an association between ETS exposure during pregnancy or at birth and risk of CD. There may be several reasons for these discrepancies. The Lashner et al, (1993) (CD=39 cases, 72 controls) and the Russell et al, (2005) (62 matched case-control pairs) studies were based on small samples, and the resulting findings could have been overestimated. Lashner et al, (1993) did not evaluate ETS exposure during pregnancy (but at birth), and did not account for potential confounding variables such as SES and family history of IBD. Similarly, Russell et al, (2005) did not adjust for potential confounding variables. The Roberts et al, (2011) study was a retrospective cohort study based on administrative data and had a sample size similar to our study (114 cases, 248479 controls). It, however, relied on record linkage and the diagnosis of CD was not confirmed. Furthermore, the data on smoking was not actively collected, but extracted from inpatient and outpatient files and missing data was a concern.

With regards to post-natal smoking, only one study investigated the associations with childhood-CD (Baron et al, 2005) and reported no associations. The reasons for the discrepancy with our study are unclear. The prevalence of passive smoke exposure during childhood in the controls was much lower in the Baron et al, (2005) study (14%) as compared to our study (42.9%), suggesting that exposure

ascertainment may have been incomplete in their study. Lack of information on the exact time window they investigated hampers adequate comparisons between these two studies.

It is important to note that none of the previous studies, in particular the ones that investigated maternal smoking during pregnancy, accounted for potential confounding from post-natal ETS exposures, and did not examine the inter-relationship between maternal and paternal exposures. This is relevant for the studies that showed an association between maternal smoking during pregnancy and an increased risk of CD in children. It is well described that families that smoke during or post-pregnancy differ from families that do not smoke (Cnattingius et al, 1992; Fingerhut et al, 1990; Glassman et al, 1990; Kahn et al, 2002; Smedberg et al, 2014). If these differences (SES, behaviour, beliefs, attitudes towards children, stress levels, etc.) are related to the predisposition of CD, then the studies could have confounded positive associations with maternal smoking. We attempted to examine the potential residual confounding by carrying out appropriate sub-group analyses. Nonetheless, the potential for residual confounding is present our findings. The association between post-natal paternal smoking and CD should be interpreted keeping these limitations in mind.

STUDY IMPACT AND FUTURE CHALLENGES

Active smoking is a known risk factor for adult-onset CD. Evidence in regards to this risk factor in childhood-onset CD, however, remains inconsistent. Our study suggests that indoor exposure to ETS during pregnancy may not increase the risk of developing CD in the child, whereas post-natal ETS exposure may be important. Although these findings need to be validated, it is clear that exposure to ETS is an issue to address not only within the context of CD, but in that of other chronic ailments such as childhood-onset asthma. Towards this end, it is relevant that legislation is currently being formulated to prevent smoking within the confines of an automobile in the presence of a child. Furthermore, the ban on smoking in public places, terraces, recreational parks, near day-care facilities, etc. is likely to reduce ETS

exposures and its associated risks in the near future. The impact of such interventions on childhood-onset CD can be topics for future research. Although ETS exposure awareness is being well implemented, re-enforcing it via continuous education will lead to long-term benefits.

REFERENCES

Abraham, B. P., S. Mehta and H. B. El-Serag (2012). "Natural History of Pediatric-onset Inflammatory Bowel Disease." Journal of Clinical Gastroenterology 46(7): 581-589.

Abramson, O., M. Durant, W. Mow, A. Finley, P. Kodali, A. Wong, V. Tavares, E. McCroskey, L. Liu, J. D. Lewis, J. E. Allison, N. Flowers, S. Hutfless, F. S. Velayos, G. S. Perry, R. Cannon and L. J. Herrinton (2010). "Incidence, Prevalence, and Time Trends of Pediatric Inflammatory Bowel Disease in Northern California, 1996 to 2006." The Journal of Pediatrics 157(2): 233-239.e231.

Al-Hawary, M. and E. M. Zimmermann (2012). "A new look at Crohn's disease." Current Opinion in Gastroenterology: 1.

Amre, DK., S. D'Souza, K. Morgan, et al (2007). "Imbalances in dietary consumption of fatty acids, vegetables, and fruits are associated with risk for Crohn's disease in children." American Journal of Gastroenterology 102(9):2016-2025.

Amre, D. K., P. Lambrette, L. Law, A. Krupoves, V. Chotard, F. Costea, G. Grimard, D. Israel, D. Mack and E. G. Seidman (2006). "Investigating the Hygiene Hypothesis as a Risk Factor in Pediatric Onset Crohn's Disease: A Case-Control Study." The American Journal of Gastroenterology 101(5): 1005-1011.

Amre, D. K. and E. G. Seidman (2003). "DNA variants in cytokine and NOD2 genes, exposures to infections and risk for Crohn's disease." Paediatr Perinat Epidemiol 17(3): 302-312.

Amre,K. D. and E. G Seidman (2003). "Etiopathogenesis of pediatric Crohn's disease. Biologic pathways based on interactions between genetic and environmental factors." Medical Hypotheses 60(3): 344-350.

Ananthakrishnan, A. N. (2015). "Epidemiology and risk factors for IBD." Nature Reviews Gastroenterology & Hepatology 12(4): 205-217.

Ananthakrishnan, A. N., D. D. Nguyen, J. Sauk, V. Yajnik and R. J. Xavier (2014). "Genetic Polymorphisms in Metabolizing Enzymes Modifying the Association Between Smoking and Inflammatory Bowel Diseases." Inflammatory Bowel Diseases 20(5): 783-789.

Aspberg, S., G. Dahlquist, T. Kahan and B. Källén (2006). "Fetal and perinatal risk factors for inflammatory bowel disease." Acta Paediatrica 95(8): 1001-1004.

Banaszkiewicz, A., A. Radzikowski and P. Albrecht (2015). "Immunisation in children and adolescents with inflammatory bowel disease." Advances in Medical Sciences 60(1): 144-147.

Baron, S. (2005). "Environmental risk factors in paediatric inflammatory bowel diseases: a population based case control study." Gut 54(3): 357-363.

Baumgart, D. C. and W. J. Sandborn (2012). "Crohn's disease." The Lancet 380(9853): 1590-1605.

Benchimol, E. I., K. J. Fortinsky, P. Gozdyra, M. Van den Heuvel, J. Van Limbergen and A. M. Griffiths (2011). "Epidemiology of pediatric inflammatory bowel disease: A systematic review of international trends." Inflammatory Bowel Diseases 17(1): 423-439.

Bergeron, V., V. Grondin, S. Rajca, M.-A. Maubert, B. Pigneur, G. Thomas, G. Trugnan, L. Beaugerie, J.

Cao S, C. Yang, Y. Gan, Z. Lu (2015). “The health effects of passive smoking. An overview of systematic reviews based on observational Epidemiological evidence.” PLos One 10(10): e0139907.

Cosnes, J. Masliah, H. Sokol, P. Seksik and M. Bachelet (2012). "Current smoking differentially affects blood mononuclear cells from patients with crohn’s disease and ulcerative colitis: Relevance to its adverse role in the disease." Inflammatory Bowel Diseases 18(6): 1101-1111.

Bernstein, C. N., P. Rawsthorne, M. Cheang and J. F. Blanchard (2006). "A Population-Based Case Control Study of Potential Risk Factors for IBD." The American Journal of Gastroenterology 101(5): 993-1002.

Bernstein, C. N., A. Wajda, L. W. Svenson, A. MacKenzie, M. Koehoorn, M. Jackson, R. Fedorak, D. Israel and J. F. Blanchard (2006). "The Epidemiology of Inflammatory Bowel Disease in Canada: A Population-Based Study." The American Journal of Gastroenterology 101(7): 1559-1568.

Biedermann, L., K. Brülisauer, J. Zeitz, P. Frei, M. Scharl, S. R. Vavricka, M. Fried, M. J. Loessner, G. Rogler and M. Schuppler (2014). "Smoking Cessation Alters Intestinal Microbiota." Inflammatory Bowel Diseases 20(9): 1496-1501.

Birrenbach, T. and U. Böcker (2004). "Inflammatory Bowel Disease and Smoking." Inflammatory Bowel Diseases 10(6): 848-859.

Bitton, A., M. Vutcovici, V. Patenaude, M. Sewitch, S. Suissa and P. Brassard (2014). "Epidemiology of Inflammatory Bowel Disease in Québec." Inflammatory Bowel Diseases 20(10): 1770-1776.

Bonen, D. (2003). "The genetics of inflammatory bowel disease." Gastroenterology 124(2): 521-536.

Bousvaros, A., F. Sylvester, S. Kugathasan, E. Szigethy, C. Fiocchi, R. Colletti, A. Otley, D. Amre, G. Ferry, S. J. Czinn, J. B. Splawski, M. Oliva-Hemker, J. S. Hyams, W. A. Faubion, B. S. Kirschner and M. C. Dubinsky (2006). "Challenges in pediatric inflammatory bowel disease." Inflammatory Bowel Diseases 12(9): 885-913.

Brant, S. R., M.-H. Wang, P. Rawsthorne, M. Sargent, L. W. Datta, F. Nouvet, Y. Y. Shugart and C. N. Bernstein (2007). "A Population-Based Case-Control Study of CARD15 and Other Risk Factors in Crohn's Disease and Ulcerative Colitis." The American Journal of Gastroenterology 102(2): 313-323.

Burgmann, T., I. Clara, L. Graff, J. Walker, L. Lix, P. Rawsthorne, C. McPhail, L. Rogala, N. Miller and C. N. Bernstein (2006). "The Manitoba Inflammatory Bowel Disease Cohort Study: Prolonged Symptoms Before Diagnosis—How Much Is Irritable Bowel Syndrome?" Clinical Gastroenterology and Hepatology 4(5): 614-620.

Canavan, C., K. R. Abrams and J. F. Mayberry (2007). "Meta-analysis: mortality in Crohn's disease." Alimentary Pharmacology & Therapeutics 25(8): 861-870.

Carbonnel, F., P. Jantchou, E. Monnet and J. Cosnes (2009). "Environmental risk factors in Crohn's disease and ulcerative colitis: an update." Gastroentérologie Clinique et Biologique 33: S145-S157.

Castiglione, F., M. Diaferia, F. Morace, O. Labianca, C. Meucci, A. Cuomo, A. Panarese, M. Romano, I. Sorrentini, C. D'Onofrio, N. Caporaso and A. Rispo (2012). "Risk factors for inflammatory bowel diseases according to the "hygiene hypothesis": A case–control, multi-centre, prospective study in Southern Italy." Journal of Crohn's and Colitis 6(3): 324-329.

Chelchowska, M., J. Ambroszkiewicz, J. Gajewska, T.Laskowska-Klita and J. Leibschang (2011). "The effect of tobacco smoking during pregnancy on plasma oxidant and antioxidant status in mother and newborn." European Journal of Obstetrics, Gynecology and Reproductive Biology 155:132–136.

Cnattingius S, G. Lindmark, O. Meirik (1992). "Who continues to smoke while pregnant?" Journal of Epidemiology and Community Health 46(3):218-221.

Dittrich, R, A. Schibel, I. Hoffmann, A. Mueller, M.W. Beckmann and S. Cupisti (2012). "Influence of maternal smoking during pregnancy on oxidant status in amniotic fluid." In Vivo 26:813–818.

Cho, J. H. and S. R. Brant (2011). "Recent Insights Into the Genetics of Inflammatory Bowel Disease." Gastroenterology 140(6): 1704-1712.e1702.

Cosnes, J. (2004). "Tobacco and IBD: relevance in the understanding of disease mechanisms and clinical practice." Best Practice & Research Clinical Gastroenterology 18(3): 481-496.

Cosnes, J., C. Gower–Rousseau, P. Seksik and A. Cortot (2011). "Epidemiology and Natural History of Inflammatory Bowel Diseases." Gastroenterology 140(6): 1785-1794.e1784.

De Cruz, P., M. A. Kamm, L. Prideaux, P. B. Allen and G. Moore (2013). "Mucosal Healing in Crohn's Disease." Inflammatory Bowel Diseases 19(2): 429-444.

de la Chica, R. A. (2005). "Chromosomal Instability in Amniocytes From Fetuses of Mothers Who Smoke." JAMA 293(10): 1212.

Di Sabatino, A., L. Rovedatti, F. Vidali, T. T. MacDonald and G. R. Corazza (2011). "Recent advances in understanding Crohn's disease." Intern Emerg Med 8(2): 101-113.

Dubois, L. and M. Girard (2005). "Breast-feeding, day-care attendance and the frequency of antibiotic treatments from 1.5 to 5 years: a population-based longitudinal study in Canada." Social Science & Medicine 60(9): 2035-2044.

Economou, M. and G. Pappas (2008). "New global map of Crohn's disease: Genetic, environmental, and socioeconomic correlations." Inflammatory Bowel Diseases 14(5): 709-720.

Eliakim, R., S. Reif, A. Lavy, D. Keter, S. Odes, A. Halak, E. Broide, Y. Niv, Y. Ron, J. Paz, A.

Farmer, R. G., W. A. Hawk and R. B. Turnbull, Jr. (1975). "Clinical patterns in Crohn's disease: a statistical study of 615 cases." Gastroenterology 68(4 Pt 1): 627-635.

Fedorak, R. N., K. Wong and R. Bridges (2010). "Canadian Digestive Health Foundation Public Impact Series. Inflammatory bowel disease in Canada: Incidence, prevalence, and direct and indirect economic impact." Can J Gastroenterol 24(11): 651-655.

Feeney, M. A., F. Murphy, A. J. Clegg, T. M. Trebble, N. M. Sharer and J. A. Snook (2002). "A case–control study of childhood environmental risk factors for the development of inflammatory bowel disease." European Journal of Gastroenterology & Hepatology 14(5): 529-534.

Ferkolj, I., P. R. Galle, A. Gangl, and B. Vucelic. Pathogenesis and Clinical Practice in Gastroenterology: Proceedings of the Falk Symposium 160 Held in Portorož, Slovenia, June 15-16, 2007. Dordrecht, The Netherlands: Springer, 2008.

Fich, Y. Villa and T. Gilat (2000). "Passive smoking in patients with inflammatory bowel disease." European Journal of Gastroenterology & Hepatology 12(9): 975-979.

Fingerhut LA, J.C. Kleinman, J.S. Kendrick (1990). "Smoking before, during, and after pregnancy." American Journal of Public Health 80(5):541-544.

Frolkis, A., L. A. Dieleman, H. W. Barkema, R. Panaccione, S. Ghosh, R. N. Fedorak, K. Madsen, G. G. Kaplan and I. B. D. C. Alberta (2013). "Environment and the inflammatory bowel diseases." Can J Gastroenterol 27(3): e18-24.

Gasche, C., J. Scholmerich, J. Brynskov, G. D'Haens, S. B. Hanauer, E. J. Irvine, D. P. Jewell, D. Rachmilewitz, D. B. Sachar, W. J. Sandborn and L. R. Sutherland (2000). "A simple classification of Crohn's disease: Report of the Working Party for the World Congresses of Gastroenterology, Vienna 1998." Inflamm Bowel Dis 6(1): 8-15.

Gauderman W, Morrison J. "A computer program for power and sample size calculations for genetic-epidemiology studies." QUANTO In. 1.1 ed; 2006

Geboes, K., J.-F. Colombel, A. Greenstein, D. P. Jewell, W. J. Sandborn, M. H. Vatn, B. Warren and R. H. Riddell (2008). "Indeterminate colitis: A review of the concept — What's in a name?" Inflammatory Bowel Diseases 14(6): 850-857.

Glassman AH, J.E. Helzer, L.S. Covey, et al (1990). "Smoking, smoking cessation, and major depression." JAMA 264(12):1546-1549.

Gomollon, F. (2014). "[Developments in the treatment of inflammatory bowel disease: 2014 overview]." Gastroenterol Hepatol 37 Suppl 3: 14-21.

Green, C. (2006). "A Population-based Ecologic Study of Inflammatory Bowel Disease: Searching for Etiologic Clues." American Journal of Epidemiology 164(7): 615-623.

Griffiths, A. M. (2004). "Specificities of inflammatory bowel disease in childhood." Best Practice & Research Clinical Gastroenterology 18(3): 509-523.

Han, D. Y., A. G. Fraser, P. Dryland and L. R. Ferguson (2010). "Environmental factors in the development of chronic inflammation: A case–control study on risk factors for Crohn's disease within New Zealand." Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis 690(1-2): 116-122.

Harries, A. D., A. Baird and J. Rhodes (1982). "Non-smoking: a feature of ulcerative colitis." BMJ 284(6317): 706-706.

Hatoum, O. A., J. Heidemann and D. G. Binion (2006). "The Intestinal Microvasculature as a Therapeutic Target in Inflammatory Bowel Disease." Annals of the New York Academy of Sciences 1072(1): 78-97.

Heikenen, J. B., S. L. Werlin, C. W. Brown and J. P. Balint (1999). "Presenting Symptoms and Diagnostic Lag in Children with Inflammatory Bowel Disease." Inflammatory Bowel Diseases 5(3): 158-160.

Hlavaty, T., J. Toth, T. Koller, A. Krajcovicova, S. Oravcova, Z. Zelinkova and M. Huorka (2013). "Smoking, breastfeeding, physical inactivity, contact with animals, and size of the family influence the risk of inflammatory bowel disease: A Slovak case-control study." United European Gastroenterology Journal 1(2): 109-119.

Jones, D. T., M. T. Osterman, M. Bewtra and J. D. Lewis (2008). "Passive Smoking and Inflammatory Bowel Disease: A Meta-Analysis." The American Journal of Gastroenterology 103(9): 2382-2393.

Jostins, L., S. Ripke, R. K. Weersma, R. H. Duerr, D. P. McGovern, K. Y. Hui, J. C. Lee, L. Philip Schumm, Y. Sharma, C. A. Anderson, J. Essers, M. Mitrovic, K. Ning, I. Cleynen, E. Theatre, S. L. Spain, S. Raychaudhuri, P. Goyette, Z. Wei, C. Abraham, J.-P. Achkar, T. Ahmad, L. Amininejad, A. N. Ananthakrishnan, V. Andersen, J. M. Andrews, L. Baidoo, T. Balschun, P. A. Bampton, A. Bitton, G. Boucher, S. Brand, C. Büning, A. Cohain, S. Cichon, M. D'Amato, D. De Jong, K. L. Devaney, M. Dubinsky, C. Edwards, D. Ellinghaus, L. R. Ferguson, D. Franchimont, K. Fransen, R. Gearry, M. Georges, C. Gieger, J. Glas, T. Haritunians, A. Hart, C. Hawkey, M. Hedl, X. Hu, T. H. Karlsen, L. Kupcinskas, S. Kugathasan, A. Latiano, D. Laukens, I. C. Lawrance, C. W. Lees, E. Louis, G. Mahy, J. Mansfield, A. R. Morgan, C. Mowat, W. Newman, O. Palmieri, C. Y. Ponsioen, U. Potocnik, N. J. Prescott, M. Regueiro, J. I. Rotter, R. K. Russell, J. D. Sanderson, M. Sans, J. Satsangi, S. Schreiber, L. A. Simms, J. Sventoraityte, S. R. Targan, K. D. Taylor, M. Tremelling, H. W. Verspaget, M. De Vos, C. Wijmenga, D. C. Wilson, J. Winkelmann, R. J. Xavier, S. Zeissig, B. Zhang, C. K. Zhang, H. Zhao, M. S. Silverberg, V. Annese, H. Hakonarson, S. R. Brant, G. Radford-Smith, C. G. Mathew, J. D. Rioux, E. E. Schadt, M. J. Daly, A. Franke, M. Parkes, S. Vermeire, J. C. Barrett and J. H. Cho (2012). "Host–microbe interactions have shaped the genetic architecture of inflammatory bowel disease." Nature 491(7422): 119-124.

Kahn RS, L. Certain, R.C. Whitaker (2002). "A reexamination of smoking before, during and after pregnancy." American Journal of Public Health 92(11):1801-1808.

Kappelman, M. D., S. L. Rifas–Shiman, K. Kleinman, D. Ollendorf, A. Bousvaros, R. J. Grand and J. A. Finkelstein (2007). "The Prevalence and Geographic Distribution of Crohn's Disease and Ulcerative Colitis in the United States." Clinical Gastroenterology and Hepatology 5(12): 1424-1429.

Karlinger, K., T. Györke, E. Makö, Á. Mester and Z. Tarján (2000). "The epidemiology and the pathogenesis of inflammatory bowel disease." European Journal of Radiology 35(3): 154-167.

Khalili, H., L. M. Higuchi, A. N. Ananthakrishnan, J. M. Richter, D. Feskanich, C. S. Fuchs and A. T. Chan (2012). "Oral contraceptives, reproductive factors and risk of inflammatory bowel disease." Gut 62(8): 1153-1159.

Kim, S. C. and G. D. Ferry (2004). "Inflammatory bowel diseases in pediatric and adolescent patients: Clinical, therapeutic, and psychosocial considerations." Gastroenterology 126(6): 1550-1560.

Lakatos, P.-L. (2007). "Smoking in inflammatory bowel diseases: Good, bad or ugly?" World Journal of Gastroenterology 13(46): 6134.

Lashner, B. A., N. J. Shaheen, S. B. Hanauer and B. S. Kirschner (1993). "Passive smoking is associated with an increased risk of developing inflammatory bowel disease in children." Am J Gastroenterol 88(3): 356-359.

Latif, E. (2012). "The Impact of Retirement on Health in Canada." Canadian Public Policy 38(1): 15-29.

Leong, R. W. L. (2010). "Environmental risk factors in inflammatory bowel disease." Journal of Gastroenterology and Hepatology 25(2): 227-228.

Lev-Tzion, R. and D. Turner (2012). "Is pediatric IBD treatment different than in adults?" Minerva Gastroenterol Dietol 58(2): 137-150.

Loftus, E. V. (2004). "Clinical epidemiology of inflammatory bowel disease: incidence, prevalence, and environmental influences." Gastroenterology 126(6): 1504-1517.

Lowe, A.-M., P.-O. Roy, M. B.-Poulin, P. Michel, A. Bitton, L. St-Onge and P. Brassard (2009). "Epidemiology of Crohn's Disease in Québec, Canada." Inflammatory Bowel Diseases 15(3): 429-435.

Mahid, S. S., K. S. Minor, A. J. Stromberg and S. Galandiuk (2007). "Active and Passive Smoking in Childhood Is Related to the Development of Inflammatory Bowel Disease." Inflammatory Bowel Diseases 13(4): 431-438.

Mahid, S. S., K. S. Minor, A. J. Stromberg and S. Galandiuk (2007). "Active and Passive Smoking in Childhood Is Related to the Development of Inflammatory Bowel Disease." Inflammatory Bowel Diseases 13(4): 431-438.

Masters, B. R. (2012). "Harrisons's Principles of Internal Medicine, 18th Edition, two volumes and DVD. Eds: Dan L. Longo, Anthony S. Fauci, Dennis L. Kasper, Stephen L. Hauser, J. Larry Jameson and Joseph Loscalzo, ISBN-13: 9780071748896 McGraw Hill." Graefes Arch Clin Exp Ophthalmol 250(9): 1407-1408.

McGilligan, V. E., J. M. W. Wallace, P. M. Heavey, D. L. Ridley and I. R. Rowland (2007). "Hypothesis about mechanisms through which nicotine might exert its effect on the interdependence of inflammation and gut barrier function in ulcerative colitis." Inflammatory Bowel Diseases 13(1): 108-115.

Mekhjian, H. S., D. M. Switz, C. S. Melnyk, G. B. Rankin and R. K. Brooks (1979). "Clinical features and natural history of Crohn's disease." Gastroenterology 77(4 Pt 2): 898-906.

Molodecky, N. A., I. S. Soon, D. M. Rabi, W. A. Ghali, M. Ferris, G. Chernoff, E. I. Benchimol, R. Panaccione, S. Ghosh, H. W. Barkema and G. G. Kaplan (2012). "Increasing Incidence and Prevalence of the Inflammatory Bowel Diseases With Time, Based on Systematic Review." Gastroenterology 142(1): 46-54.e42.

Morrison, G., B. Headon and P. Gibson (2009). "Update in inflammatory bowel disease." Aust Fam Physician 38(12): 956-961.

Moum, B. (2003). "Inflammatory Bowel Disease. Diagnosis and Therapeutics. Edited by Russell D. Cohen (Totowa, NJ: Humana Press, 2003). 350 pp. Hardcover." Scand J Gastroenterol 38(9): 1012-1012.

Munyaka, P. M., E. Khafipour and J.-E. Ghia (2014). "External Influence of Early Childhood Establishment of Gut Microbiota and Subsequent Health Implications." Frontiers in Pediatrics 2.

Ng, S. C., C. N. Bernstein, M. H. Vatn, P. L. Lakatos, E. V. Loftus, C. Tysk, C. O'Morain, B. Moum and J. F. Colombel (2013). "Geographical variability and environmental risk factors in inflammatory bowel disease." Gut 62(4): 630-649.

Parkes, G. C., K. Whelan and J. O. Lindsay (2014). "Smoking in inflammatory bowel disease: Impact on disease course and insights into the etiology of its effect." Journal of Crohn's and Colitis 8(8): 717-725.

Persson, P. G., A. Ahlbom and G. Hellers (1990). "Inflammatory bowel disease and tobacco smoke--a case-control study." Gut 31(12): 1377-1381.

Petersen, B.-S., M. E. Spehlmann, A. Raedler, B. Stade, I. Thomsen, R. Rabionet, P. Rosenstiel, S. Schreiber and A. Franke (2014). "Whole genome and exome sequencing of monozygotic twins discordant for Crohn's disease." BMC Genomics 15(1): 564.

Pimentel, M., M. Chang, E. J. Chow, S. Tabibzadeh, V. Kirit-Kiriak, S. R. Targan and H. C. Lin (2000). "Identification of a prodromal period in Crohn's disease but not ulcerative colitis." The American Journal of Gastroenterology 95(12): 3458-3462.

Poullenot, F. and D. Laharie (2014). "[First line therapy of inflammatory bowel disease]." Rev Prat 64(9): 1242-1248.

Public Health Agency of Canada. Perinatal Health indicators for Canada 2013. A report of the Canadian Perinatal Surveillance System, Ottawa 2013.

Rabizadeh, S. and M. Dubinsky (2013). "Update in Pediatric Inflammatory Bowel Disease." Rheumatic Disease Clinics of North America 39(4): 789-799.

Razani-Boroujerdi, S., R. T. Boyd, M. I. Davila-Garcia, J. S. Nandi, N. C. Mishra, S. P. Singh, J. C. Pena-Philippides, R. Langley and M. L. Sopori (2007). "T Cells Express 7-Nicotinic Acetylcholine Receptor Subunits That Require a Functional TCR and Leukocyte-Specific Protein Tyrosine Kinase for Nicotine-Induced Ca2+ Response." The Journal of Immunology 179(5): 2889-2898.

Reif, S., A. Lavy, D. Keter, A. Fich, R. Eliakim, A. Halak, E. Broide, Y. Niv, Y. Ron, J. Patz, S. Odes, Y. Villa and T. Gilat (2000). "Lack of association between smoking and Crohn's disease but the usual association with ulcerative colitis in Jewish patients in Israel: a multicenter study." The American Journal of Gastroenterology 95(2): 474-478.

Richardson, C. E. (2003). "Effect of smoking and transdermal nicotine on colonic nicotinic acetylcholine receptors in ulcerative colitis." QJM 96(1): 57-65.

Rigas, A., B. Rigas, M. Glassman, Y.-Y. Yen, S. J. Lan, E. Petridou, C.-C. Hsieh and D. Trichopoulos (1993). "Breast-feeding and maternal smoking in the etiology of Crohn's disease and ulcerative colitis in childhood." Annals of Epidemiology 3(4): 387-392.

Roberts, S. E. (2011). "Perinatal and early life risk factors for inflammatory bowel disease." World Journal of Gastroenterology 17(6): 743.

Rocchi, A., E. I. Benchimol, C. N. Bernstein, A. Bitton, B. Feagan, R. Panaccione, K. W. Glasgow, A. Fernandes and S. Ghosh (2012). "Inflammatory bowel disease: a Canadian burden of illness review." Can J Gastroenterol 26(11): 811-817.

Rossiter, C. E. and J. J. Schlesselman (1983). "Case-Control Studies. Design, Conduct, Analysis." Biometrics 39(3): 821.

Russell, R. K., R. Farhadi, M. Wilson, H. Drummond, J. Satsangi and D. C. Wilson (2005). "Perinatal passive smoke exposure may be more important than childhood exposure in the risk of developing childhood IBD." Gut 54(10): 1500-1501; author reply 1501.

Sahinli, A.S, K. Marakoğlu and A. Kiyici (2012). “Evaluation of the levels of oxidative stress factors and ischemia modified albumin in the cord blood of smoker and non-smoker pregnant women.” Journal of Maternal, Fetal and Neonatal Medicine 25:1064–1068.

Sandler, R. S., D. P. Sandler, C. W. McDonnell and J. I. Wurzelmann (1992). "Childhood exposure to environmental tobacco smoke and the risk of ulcerative colitis." Am J Epidemiol 135(6): 603-608.

Schein, M. (2010). "Schwartz’s Principles of Surgery, F. Charles Brunicardi, Dana K. Andersen, Timothy R. Billiar, David L. Dunn, John G. Hunter, Jeffrey B. Matthews, Raphael E. Pollock (Eds)." World J Surg 34(4): 871-873.

Seong MW, J.H. Hwang, J.S. Moon, H.J. Ryu, S.Y. Kong, T.H. Um (2008). “Neonatal hair nicotine levels and fetal exposure to paternal smoking at home.” American Journal of Epidemiololgy 168:1140-4.

Silverberg, M. S., J. Satsangi, T. Ahmad, I. D. Arnott, C. N. Bernstein, S. R. Brant, R. Caprilli, J. F. Colombel, C. Gasche, K. Geboes, D. P. Jewell, A. Karban, E. V. Loftus, Jr., A. S. Pena, R. H. Riddell, D. B. Sachar, S. Schreiber, A. H. Steinhart, S. R. Targan, S. Vermeire and B. F. Warren (2005). "Toward an integrated clinical, molecular and serological classification of inflammatory bowel disease: report of a Working Party of the 2005 Montreal World Congress of Gastroenterology." Can J Gastroenterol 19 Suppl A: 5A-36A.

Smedberg J, A. Lupattelli, A.C. Mardby, et al (2014). “Characteristics of women who smoke during pregnancy: a cross-sectional study of pregnant women and new mothers in 15 different European countries.” BMC Pregnancy and Childbirth 14:213.

Sobczak, M., A. Fabisiak, N. Murawska, E. Wesołowska, P. Wierzbicka, M. Wlazłowski, M. Wójcikowska, H. Zatorski, M. Zwolińska and J. Fichna (2014). "Current overview of extrinsic and intrinsic factors in etiology and progression of inflammatory bowel diseases." Pharmacological Reports 66(5): 766-775.

Spalinger, M. R., G. Rogler and M. Scharl (2014). "Crohn's Disease: Loss of Tolerance or a Disorder of Autophagy?" Dig Dis 32(4): 370-377.

Springmann V, P. Brassard, A. Krupoves, et al (2014). "Timing, frequency and type of physician-diagnosed infections in childhood and risk for Crohn’s disease in children and young adults." Inflammatory Bowel Diseases 20(8):1346-1352.

Statistics Canada- Health Statistics Division. Canadian Community Health Survey (CCHS) Cycle 4.1 2007-2008. 2009. Ottawa: Statistics Canada.

Statistics Canada. CANSIM- Table 102-0531- Deaths, by cause, Chapter XI: Diseases of the digestive system (K00 to K93), age group and sex, Canada, annual (2000-2006).

Szigethy, E., L. McLafferty and A. Goyal (2011). "Inflammatory Bowel Disease." Pediatric Clinics of North America 58(4): 903-920.

Thomas, Shane A., and Stephen Polgar. Introduction to Research in the Health Sciences. London: Churchill Livingstone., 2008.

Thompson, N. P., R. E. Pounder and A. J. Wakefield (1995). "Perinatal and childhood risk factors for inflammatory bowel disease: a case-control study." European Journal of Gastroenterology and Hepatology 7(5): 385-390.

Tong VT, J.R. Jones, P.M. Dietz, D. D'Angelo, J.M. Bombard (2009). "Trends in smoking before, during and after pregnancy- Pregnancy risk assessment monitoring system (PRAMS), United States, 31 cities, 2000-2005." Mortality and morbidity weekly report surveillance summaries 58(4):1-29.

Tysk, C., E. Lindberg, G. Jarnerot and B. Floderus-Myrhed (1988). "Ulcerative colitis and Crohn's disease in an unselected population of monozygotic and dizygotic twins. A study of heritability and the influence of smoking." Gut 29(7): 990-996.

Van Limbergen, J. and A. M. Griffiths (2010). "Pediatric Inflammatory Bowel Disease in the Emergency Department." Clinical Pediatric Emergency Medicine 11(3): 189-197.

Vermeire, S., G. Van Assche and P. Rutgeerts (2012). "Classification of inflammatory bowel disease." Current Opinion in Gastroenterology 28(4): 321-326.

Warren CW, N.R. Jones, A. Peruga, J. Chauvin, J.P. Baptiste, V.C. de Silva (2008). Global Youth Tobacco Surveillance 2000-2007. Morbidity and Mortality Weekly Report 57(SS01):1-21.

Yang S, A. Decker, M.S. Kramer (2013). "Exposure to parental smoking and child growth and development. A cohort study." BMC Pediatrics 2013;13:104.

Ziv, Y., A. Nevler, E. Willenz, O. Doron, A. Zbar, A. Shperber and J. Sandbank (2015). "A novel porcine model for chemically inducible Crohn's-like reaction." Israel Medical Association Journal 17(1): 19-23.

APPENDIX 1: STUDY DESIGN

At study commencement (June 2013)

CD case pool (~500)

Select a case newly diagnosed in 2012
(with a diagnosis confirmed after at least 1 year of follow-up)

Contact and invite the case for participation in the study and provide
Information on ETS exposure

Once case is included, move to the pool of controls

Control pool (~500)

Identify a potential control who had visited the clinic (orthopedic or gastroenterology) within ± 3 months of the diagnosis of the case and who resides in the same geographic area as the case (based on the first 3 digits of the postal code)

Contact and invite for participation in ETS study

Move to the next case and follow the same strategy
Once cases from 2012 were exhausted, move on to cases diagnosed in 2011 and carry out the same strategy for identification, selection & recruitment

During study period (June 2013 to Jan 2015)

Identify cases who were newly diagnosed and with a diagnosis
Confirmed after a minimum follow-up of 1 year
Contact and invite the case for participation in the study and provide
Information on ETS exposure

Once case is included, move to the pool of controls

Identify potential controls recruited during the study period, contact, invite and recruit as above

APPENDIX 2

ENVIRONMENTAL TOBACCO SMOKE EXPOSURE QUESTIONNAIRE

Study ID		**Contact date**	
Date of diagnosis(case)		**Date of first interview (control group)**	
Family history of IBD		**Maternal education**	
Ethnicity		**Paternal education**	

Date of diagnosis of Crohn's Disease (date of first interview for control group):

EXPOSURE DURING PREGNANCY:

1. **Have you ever smoked tobacco?**

   ◯ Yes ◯ No ◯ Refused ◯ Don't know

   (For interviewer: If NO/REFUSED, go to Q 7…; if YES, go to next Q 2…)

2. **Did you smoke tobacco at any time when you became pregnant with (mention child's name)?**

   ◯ Yes ◯ No ◯ Refused ◯ Don't know

   (For interviewer: If NO/REFUSED, go to **Q 6**; if YES, go to **next Q**…)

3. **Did you smoke tobacco throughout (entire) your pregnancy with your child?**

   ◯ Yes ◯ No ◯ Refused ◯ Don't know

   (For interviewer: If NO, go to **next Q 4**; if YES, go to **Q 5**)

4. Did you smoke during (Interviewer: name the trimesters one by one, if necessary explain what they are)

◯ T1 ◯ T2 ◯ T3

5. On average, how many cigarettes PER DAY did you smoke during this pregnancy?

◯ 1-5 ◯ 6-10 ◯ >10 ◯ Refused ◯ Don't know

6. During this period, did you ever use nicotine patches, nicotine gum or tobacco in any other form? (As snuff or chewable form)?

◯ Yes ◯ No ◯ Refused ◯ Don't know

7. During this period, was there anyone that smoked tobacco in your vicinity (except Home):

◯ No

◯ Yes

If yes, was it ◯ Every day

◯ Some days

◯ Only on some occasions (example when walking on the street)

How many cigarette/day…….

8. During this period, did you or any other household member smoke in the house?

◯ No

◯ Yes

9. If yes, to your knowledge was it from:

- Mother ◯ Yes ◯ No
- Father ◯ Yes ◯ No
- Other people ◯ Yes, who……◯ No
- Other people ◯ Yes, who……◯ No

- Other people ◯ Yes, who……..◯ No
- Other people ◯ Yes, who……..◯ No

10. If mother, was it every day ◯

Some days ◯

Don't know ◯

Refused ◯

Average of cigarette/day……..

11. If father, was it every day ◯

Some days ◯

Don't know ◯

Refused ◯

Average of cigarette/day……..

12. If other members:

a. **How many members smoked?** Number.....

b. **Which members smoked?**.....

❖ **If other members, was it:**

Every day ◯

Some days ◯

Don't know ◯

Refused ◯

Average of cigarette/day……..

❖ **If other members, was it:**

Every day ◯

Some days ◯

Don't know ◯

Refused ◯

Average of cigarette/day……..

❖ **If other members, was it:**

Every day ◯

Some days ◯

Don't know ◯

Refused ◯

Average of cigarette/day……..

❖ **If other members, was it:**

Every day ◯

Some days ◯

Don't know ◯

Refused ◯

Average of cigarette/day……..

SMOKING DURING BREASTFEEDING OR LACTATION:

13. Did you breastfeed your child?

◯ Yes ◯ No ◯ Refused ◯ Don't know

14. If yes, for how long did you breastfeed?

_____ (number of months)

15. Did you smoke tobacco at any time during your breastfeeding with your child?

◯ Yes ◯ No ◯ Refused ◯ Don't know

16. Did you smoke tobacco throughout your breastfeeding?

◯ Yes ◯ No ◯ Refused ◯ Don't know

17. At this period, did you use alternative methods such as nicotine paths, or gum or chewable tobacco?

◯ Yes ◯ No ◯ Refused ◯ Don't know

18. On average, how many cigarettes (or tobacco in any other form) PER DAY did you smoke during this period?

◯ 1-5 ◯ 6-10 ◯ >10 ◯ Refused ◯ Don't know

SMOKING STATUS OF HOUSEHOLD MEMBER SINCE BIRTH OF CHILD TO THE INDEX DATE (DATE OF DIAGNOSIS FOR CASE & DATE OF INTERVIEW FOR CONTROL)

19. How many members (on average) were living in the household during this period?

Number of people:……

20. To your knowledge was (mention the child's name) exposed to tobacco smoke during this time period?

◯ No ◯ Yes

21. Was the exposure to tobacco smoke in the house where (mention child's name) was living most of the time?

◯ Yes ◯ No ◯ Don't know

22. If yes, to your knowledge was it from:

- Mother ◯ Yes ◯ No
- Father ◯ Yes ◯ No
- Other people ◯ Yes ◯ No……
- Other people ◯ Yes ◯ No……
- Other people ◯ Yes ◯ No……
- Other people ◯ Yes ◯ No……

23. If mother, was it every day ◯

Some days ◯

Don't know ◯

Refused ◯

Average of cigarette/day……..

24. If father, was it every day ◯

Some days ◯

Don't know ◯

Refused ◯

Average of cigarette/day……..

25. If other members:

a. **How many members smoked?** Number.....

b. **Which members smoked?**.....

- **If other members, was it**: every day ◯

Some days ◯

Don't know ◯

Refused ◯

Average nb of cigarettes/day……..

- **If other members, was it** every day ◯

Some days ◯

Don't know ◯

Refused ◯

Average nb of cigarettes/day……..

- **If other members, was it** every day ◯

Some days ◯

Don't know ◯

Refused ◯

Average nb of cigarettes/day……..

- **If other members, was it** every day ◯

Some days ◯

Don't know ◯

Refused ◯

Average nb of cigarettes/day……..

WE HAVE NOW COME TO THE END OF THE QUESTIONNAIRE. WE THANK YOU FOR YOUR PARTICIPATION

APPENDIX 3

MODEL CREATION AND DIAGNOSTICS

The a priori selected potential confounders lead to the creation of a preliminary model according to the following formula:

$$\text{logit}(P,X) = \alpha + \beta E + \sum_{i=1}^{n} \gamma_i V_i$$

comprising of the intercept, the main exposure and its coefficient, as well as all potential confounders retained and their coefficients.

The full model, at this stage, was the following:

$$\text{Logit } P(X) = \alpha + \beta\,(\text{ETS}) + \gamma_1(\text{age}) + \gamma_2(\text{family history})$$

Where: ETS=environmental tobacco smoke exposure variable, age=age at diagnosis for cases and recruitment for controls (continuous). Family history was binary.

ASSESSMENT OF LINEARITY OF CONTINUOUS VARIABLES

At this stage, the continuous variables were assessed for linearity, to decide whether they should be kept as continuous variables or not (an assumption of the logistic model). The Box-Tidwell test for linearity was implemented. A significant p-value for the test suggests that the assumption of linearity is rejected and that the covariate is not linear in the logit of the outcome and needs to be appropriately transformed.

The results of the linearity assumption test for 'AGE' are presented below:

```
boxtid logit case_cont age

------------------------------------------------------------------------------
  case_cont |      Coef.   Std. Err.      z    P>|z|     [95% Conf. Interval]
------------+-----------------------------------------------------------------
   Iaged__1 |    18.4975    409.451     0.05   0.964    -784.0116    821.0067
   Iaged_p1 |   .0113975   14.13167     0.00   0.999    -27.68617    27.70896
      _cons |   .0677129   .1317889     0.51   0.607    -.1905886    .3260144
```

```
------------------------------------------------------------------------------
age |   .0699614   .0289665      2.415   Nonlin. dev. 1.067   (P = 0.302)
 p1 |   .0340205   .3973019      0.086
------------------------------------------------------------------------------

Deviance:  357.476.

---------------------------------------
```

The p-value is 0.30 and non-significant indicating that the linearity assumption is appropriate and that age can be included as a continuous variable in the model.

ASSESSING MODEL SPECIFICATION

The specificity of each fitted model was assessed, using the LINKTEST function in STATA. As the _hat value represents the predictive power of the model, a significant result points to a well-specified model. The _hatsq function should not be significant. An example of the fitted model assessing the association between post-natal paternal smoking among mothers who never smoked, after adjusting for pre-natal paternal smoking, age and family history of IBD, and risk for CD is presented below.

```
. xi: logistic case_cont   f_post_vs_no_m_none  f_pre_vs_no_m_none agediag famhist

Logistic regression                               Number of obs   =        194
                                                  LR chi2(4)      =      10.82
                                                  Prob > chi2     =     0.0287
Log likelihood = -128.80394                       Pseudo R2       =     0.0403

------------------------------------------------------------------------------
   case_cont | Odds Ratio   Std. Err.      z    P>|z|     [95% Conf. Interval]
-------------+----------------------------------------------------------------
f_post_vs_~e |   2.748897   1.707717     1.63   0.104     .8135057    9.288733
f_pre_vs_n~e |   .9521258   .5152157    -0.09   0.928     .3296788    2.749779
     agediag |   1.050643   .0346036     1.50   0.134     .9849641    1.120702
     famhist |   1.727183   .6489214     1.45   0.146     .8270521    3.606982
------------------------------------------------------------------------------

. linktest

Iteration 0:   log likelihood = -134.21271
Iteration 1:   log likelihood = -128.60378
Iteration 2:   log likelihood =  -128.5929
Iteration 3:   log likelihood =  -128.5929

Logistic regression                               Number of obs   =        194
                                                  LR chi2(2)      =      11.24
                                                  Prob > chi2     =     0.0036
Log likelihood =  -128.5929                       Pseudo R2       =     0.0419

------------------------------------------------------------------------------
   case_cont |      Coef.   Std. Err.      z    P>|z|     [95% Conf. Interval]
-------------+----------------------------------------------------------------
        _hat |   1.058793   .3288491     3.22   0.001      .414261    1.703326
      _hatsq |  -.3287497   .5017629    -0.66   0.512    -1.312187    .6546875
       _cons |   .0824802   .1979398     0.42   0.677    -.3054747     .470435
------------------------------------------------------------------------------
```

The _hat is significant and the _hatsq is not, suggesting that the model is appropriately specified

(f_post_vs_no_m_none: Father smoking after pregnancy versus not smoking, among mothers who never smoked; f_pre_vs_no_m_none: Father smoking during pregnancy versus not smoking, among mothers who never smoked; agediag: age at diagnosis of cases and at interview of controls; famhist: Family history of IBD in first degree relatives

---

COLLINEARITY

Multicollinearity occurs when a variable in the model can be predicted from another variable in the model. This causes unreliable regression coefficients, and large variances. In order to assess multicollinearity, the variances of the coefficients were compared. An abnormally large variance for one of the coefficients points to possible multicollinearity. As no coefficient displayed an abnormally high variance, no multicollinearity issues between variables were found.

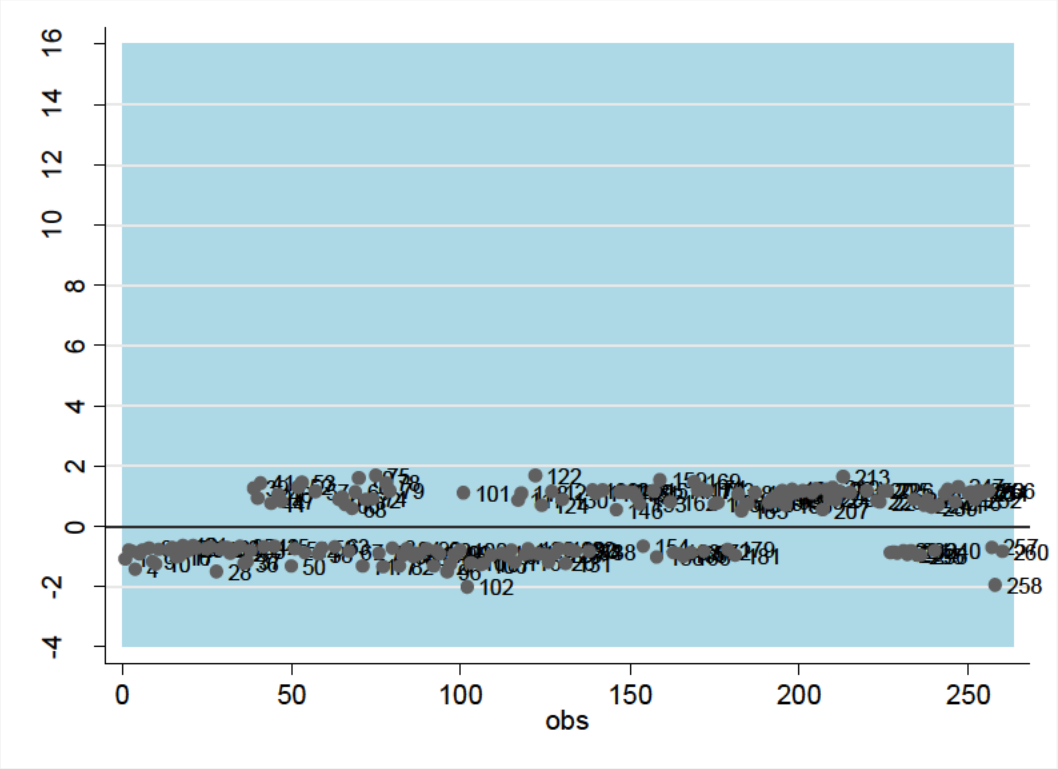
EXTREME AND INFLUENTIAL OBSERVATIONS

In this final stage of model building, the observations were assessed to detect those with extreme values and those which could have had a significant impact on the coefficients of the variables in the model.

Outliers were assessed by plotting the residuals for a particular fitted model against the observations in the data. Pearson residuals are defined to be the standardized difference between the observed frequency and the predicted frequency. They measure the relative deviations between the observed and fitted values. Deviance residual is another type of residual. It measures the disagreement between the maxima of the observed and the fitted log likelihood functions. Since logistic regression uses the maximal likelihood principle, the goal in logistic regression is to minimize the sum of the deviance residuals. Another statistic, sometimes called the hat diagonal, since technically it is the diagonal of the hat matrix, measures the leverage of an observation. It is also sometimes called the Pregibon leverage. These three statistics, Pearson residual, deviance residual and Pregibon leverage are considered to be the three basic

building blocks for logistic regression diagnostics. Below we show the application of one of these diagnostics to the model fitted above.

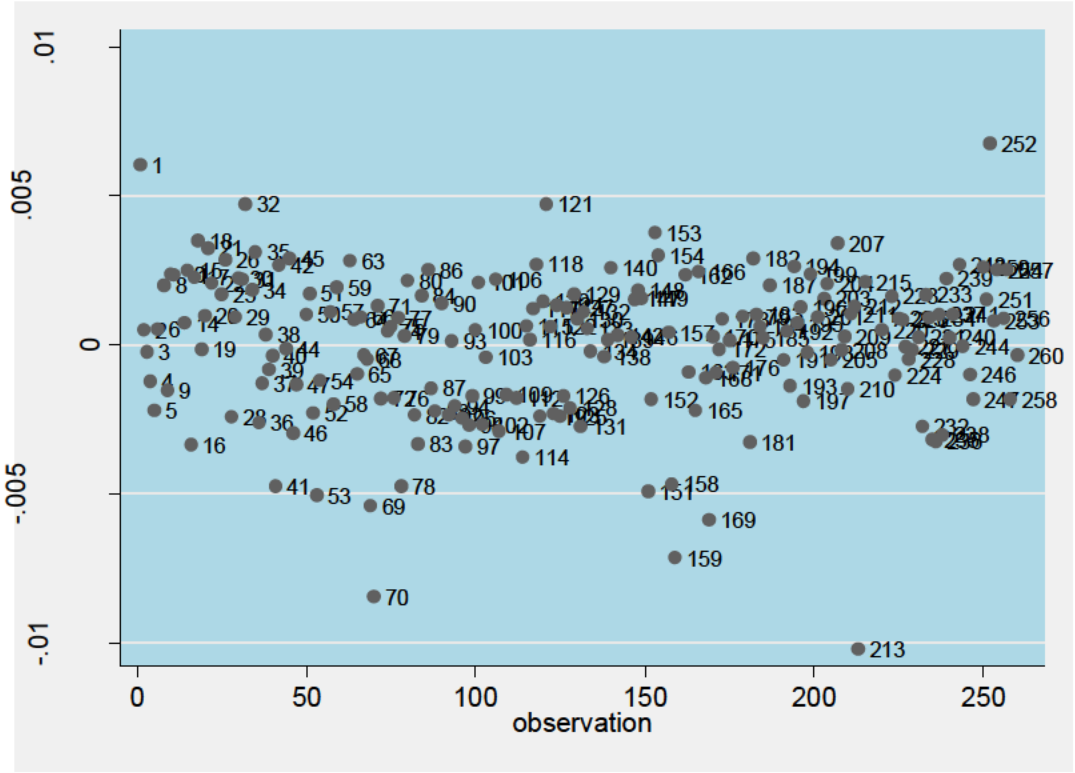
Standardized residuals versus the observations



This plot suggests that few observations deviate from the general pool in terms of residuals. However, most have values within the suggested diagnostic cut-offs (±2 for the residuals). In this model, these observations were retained.

INFLUENTIAL OBSERVATIONS

The Df beta values (a measure of the change that occurs in the coefficients in the model if the observations were removed) for each observation were estimated for the model. The outliers were identified and investigated. The dfbeta command in STATA can be given post logistic model fitting for each covariate in the model. Below is the estimation of this statistic for the covariate age in the above model



It seems observation 213 could have a higher impact. The logistic regression was run, omitting this observation. The purpose was to evaluate whether this extreme observation significantly influenced the regression coefficient. No major differences were observed and the observation was retained.

APPENDIX 4: Power calculations

Model # 1

Outcome: Disease

Design: Unmatched case-control (1:1)

Hypothesis: Environment only

Desired power: 0.800000

Significance: 0.050000, 2-sided

Binary environmental factor

Prevalence: 0.1500

Disease model Summary parameters

P0 0.000100 *kP 0.000107

RE: 1.5000 (*indicates calculated value)

RE	N Environment	kP
1.5000	655	0.000107
1.6000	478	0.000109
1.7000	369	0.000110
1.8000	296	0.000112
1.9000	244	0.000113
2.0000	206	0.000115

N is the number of cases required for the desired power

The required number of controls is 1xN

Model # 2

Outcome: Disease

Design: Unmatched case-control (1:1)

Hypothesis: Environment only

Desired power: 0.800000

Significance: 0.050000, 2-sided

Binary environmental factor

Prevalence: 0.2000

Disease model Summary parameters

P0 0.000100 *kP 0.000110

RE: 1.5000 (*indicates calculated value)

RE	Environment	kP
1.5000	534	0.000110
1.6000	391	0.000112
1.7000	303	0.000114
1.8000	244	0.000116
1.9000	202	0.000118
2.0000	171	0.000120
2.1000	148	0.000122
2.2000	130	0.000124
2.3000	115	0.000126
2.4000	104	0.000128
2.5000	94	0.000130

N is the number of cases required for the desired power, the required number of controls is 1xN

Model # 3

Outcome: Disease

Design: Unmatched case-control (1:1)

Hypothesis: Environment only

Desired power: 0.800000

Significance: 0.050000, 2-sided

Binary environmental factor

Prevalence: 0.2500

Disease model Summary parameters

P0 0.000100 *kP 0.000112

RE: 1.5000 (*indicates calculated value)

RE	N Environment	kP
1.5000	466	0.000112
1.6000	343	0.000115
1.7000	266	0.000117
1.8000	215	0.000120
1.9000	179	0.000122
2.0000	152	0.000125
2.1000	132	0.000127
2.2000	116	0.000130
2.3000	103	0.000132
2.4000	93	0.000135
2.5000	85	0.000137

N is the number of cases required for the desired power, the required number of controls is 1xN

Model # 4

Outcome: Disease

Design: Unmatched case-control (1:1)

Hypothesis: Environment only

Desired power: 0.800000

Significance: 0.050000, 2-sided

Binary environmental factor

Prevalence: 0.3000

Disease model Summary parameters

P0 0.000100 *kP 0.000115

RE: 1.5000 (*indicates calculated value)

RE	N Environment	kP
1.5000	425	0.000115
1.6000	314	0.000118
1.7000	244	0.000121
1.8000	198	0.000124
1.9000	165	0.000127
2.0000	141	0.000130
2.1000	123	0.000133
2.2000	108	0.000136
2.3000	97	0.000139
2.4000	87	0.000142
2.5000	80	0.000145

N is the number of cases required for the desired power, the required number of controls is 1xN